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THE BIG VEIN DISEASE OF LETTUCE IN RELATION TO SOIL MOISTURE[1]

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INTRODUCTION

Two diseases of unknown cause, big vein and brown blight, have been closely associated with the culture of lettuce (*Lactuca sativa* L.) in parts of California and Arizona since about 1917. Big vein has also been reported in certain places along the Atlantic seaboard.[3] The development of strains resistant to brown blight,[4] with the subsequent elimination of this disease from most commercial lettuce fields, has brought increased prominence to the less serious but as yet uncontrolled big vein. The only detailed published investigation of the disease was made by Jagger and Chandler.[5] According to their results the amount of disease was not reduced by soil applications of various major and minor elements or by prolonged leaching with water, but partial sterilization with steam or formaldehyde eliminated the causal agent. They also found that the disease could not be readily transmitted except through the soil and that it appeared to be influenced considerably by environment.

From the available data on big vein three lines of research, each contributing toward a solution of the problem, seem logical. These are (1) a determination of the identity of the causal agent and its relation to the plant, (2) a study of the environmental factors affecting the development of the disease, and (3) a search for resistant strains of lettuce. Although knowledge of the causal agent is desirable, at present it does not seem absolutely essential since another obscure disease, brown blight, has been controlled through the use of resistant varieties though its cause is not known.[6] Inasmuch as transmission has been obtained only through infective soil, and temperature and soil moisture have been suggested as important in the production of symptoms,[7] the influence of environment must be understood before satisfactory resistance trials can be instituted. As one phase of this investigation a study was made of soil moisture in relation to big vein. The results are reported in the present paper.

[1] Received for publication October 19, 1942.

[2] The writer wishes to thank W. M. Owen, formerly field aide, Division of Fruit and Vegetable Crops and Diseases, for technical assistance throughout the course of this investigation.

[3] THOMPSON, R. C., and DOOLITTLE, S. P. INFLUENCE OF TEMPERATURE ON THE EXPRESSION OF BIG-VEIN SYMPTOMS IN LETTUCE. Phytopathology 32: 542-544, illus. 1942.

[4] JAGGER, I. C. BROWN BLIGHT OF LETTUCE. Phytopathology 30: 53-64, illus. 1940.

[5] JAGGER, I. C., and CHANDLER, N. BIG VEIN, A DISEASE OF LETTUCE. Phytopathology 24: 1253-1256, illus. 1934.

[6] See footnotes 4 and 5.

[7] See footnote 5.

MATERIALS AND METHODS

Lots of infested soil from lettuce-growing districts near El Centro in the Imperial Valley of California and near Salinas, Calif., were employed in all the experiments reported herein. The experiments were conducted at Torrey Pines near La Jolla, Calif. Both soils are of fine texture and have a moisture-holding capacity of about 50 percent.[8] One-gallon glazed crocks were filled with equal amounts of soil, and water was added through a central ⅜-inch pipe, slotted at ½-inch intervals, to bring the moisture to 35, 45, 55, 70, and 85 percent of the moisture-holding capacity in the different series. The pots were frequently brought up to weight, but when transpiration was unusually high, it was sometimes necessary to carry the two lower moisture levels slightly overweight so that the plants in these series would remain alive. When it was desired to grow successive crops of lettuce on the same lot of soil, the soil was removed from the pots, mixed thoroughly, and fertilized, the moisture-holding capacity was redetermined, and the soil was replaced in the pots and brought to the desired moisture level before replanting it.

It is recognized that these methods have certain very definite limitations. For example, Hendrickson and Veihmeyer [9] have shown that adding a small amount of water to the top of soil in a container saturates the upper part to field capacity, while the lower part remains in its original dry state for long periods. Without rather elaborate equipment, which was not entirely suited to the present work, it was not possible to maintain a moisture-holding capacity of a given percentage throughout the unit volume of soil used. However, the amount of water supplied over the growing period employed was regulated with some degree of accuracy even though moisture distribution in the soil was not so uniform as might be desired. Had the amount of water in the soil that was held at 35 percent of the moisture-holding capacity been evenly distributed, it is likely that sufficient moisture would not have been present for plant growth. Also, if the moisture-holding capacity had been determined on undisturbed field soil, the figure undoubtedly would have been different from the one given. These facts should be kept in mind when considering the effect of moisture on big vein in the present work.

Two noncommercial strains of lettuce were used because of their resistance to downy mildew, which would have interfered with studies on susceptible strains. Since the two strains do not differ in susceptibility to big vein, they are treated as one variety in the data reported. Seed was sown in soil from areas where big vein does not occur, and when one or two true leaves had unfolded on the plants, four plants were transplanted to each pot. The pots were placed on an outdoor bench which could be sheltered when there was danger of rain. In each experiment the pots for the different moisture levels were randomized in 10 blocks so that variance analyses [10] of the results could be made.

[8] RIKER, A. J., and RIKER, R. S. INTRODUCTION TO RESEARCH ON PLANT DISEASES. A GUIDE . . . 117 pp., illus. St. Louis, Mo. 1936.

[9] HENDRICKSON, A. H., and VEIHMEYER, F. J. MOISTURE IN SOIL IN CONTAINERS. Plant Physiology 16: 821–826, illus. 1941.

[10] SNEDECOR, GEORGE W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 3, 422 pp., illus. Ames, Iowa. 1940.

As big vein appeared on the plants, the date and the number of leaves affected were recorded. Experiments were discontinued after 2 to 3 months when the plants at higher moisture levels were becoming crowded. Crowding usually became noticeable just before the plants began to head. At the termination of the experiments, the tops were weighed to the nearest 0.1 gm. In some experiments the top weights were tabulated in the order in which the plants in each of the crocks became diseased, so that the relation between time of symptom appearance and final weight could be determined. This procedure was followed to learn whether the disease reduces weight perceptibly during a period roughly about half of the growing season under field conditions.

EXPERIMENTAL RESULTS

EFFECT OF SOIL MOISTURE ON SYMPTOM PRODUCTION

Symptoms of big vein were complicated in a few instances by the appearance of small, mottled plants, the leaves of which were often deformed and tended to curl downward at the margins. This type of abnormality, termed "rosette," was often found on plants growing in soil where big vein occurred, but sometimes it was found on plants growing in soil where this disease did not occur. In the writer's experiments these rosette plants were not considered as having big vein unless they showed the typical vein clearing and banding.

Big vein in some representative leaves is shown in figure 1, *A*. The plants in the lower moisture series, particularly those at 35 percent of the moisture-holding capacity, were generally darker green than those in the higher moisture groups, and the symptoms often were not distinct because vein clearing and lack of chlorophyll along the veins were less pronounced than in plants supplied with more water. Plants in the four-leaf stage or older and leaves of almost any age became diseased in these experiments, but generally the most typical symptoms were found on leaves of intermediate age. In the oldest leaves big vein was often very faint or absent, and in the youngest leaves, which were still somewhat curled, the disease was seldom evident. From the leaf on which initial symptoms appeared, the disease usually extended more rapidly to the younger leaves than to the older. Vein clearing and banding often varied in severity from time to time, apparently being influenced by environmental conditions other than soil moisture. Temperature is undoubetdly very important.[11]

The statistical significance of differences between adjacent moisture percentages changed from experiment to experiment and with the various lots of soil employed. For example, in one experiment with Salinas soil the number of big vein plants occurring at 35 percent of moisture-holding capacity was significantly less than at any other moisture level. In another experiment with the same soil, the disease incidence at 35 percent was significantly less than that at 55, 70, or 85 percent, while the 45-percent group produced significantly fewer diseased plants than the 70- or 85-percent series. Considering this variation, it seemed more important from a practical standpoint to establish the over-all effect of moisture on disease incidence than to

[11] THOMPSON, R. C., and DOOLITTLE, S. P. See footnote 3, p. 1.
JAGGER, I. C., and CHANDLER, N. See footnote 5, p. 1.

FIGURE 1.—*A*, Big vein symptoms on representative leaves from plants grown on soil of different moisture levels: *a*, 35 percent of moisture-holding capacity; *b*, 45 percent; *c*, 55 percent; *d*, 70 percent; and *e*, 85 percent. The leaves in *a*, *b*, and *c* show the usual type of vein clearing and banding and those in *d* and *e* show a more extensive chlorophyll deficiency along larger veins, a symptom of rare occurrence. *B*, Comparative sizes of plants grown at the respective moisture levels explained in *A*.

determine differences between individual levels, which in these experiments were composed of rather small moisture increments. With this in mind the results from several experiments have been recorded graphically and are shown in figure 2.[12]

An examination of figure 2 shows that soil moisture profoundly affected the number of big vein plants, the percentage tending to increase with an increase in the supply of water. There was a wide divergence between the two soils at moisture levels of 45, 55, and 70 percent of the moisture-holding capacity, the Imperial Valley soil having more diseased plants at these levels than the Salinas soil.

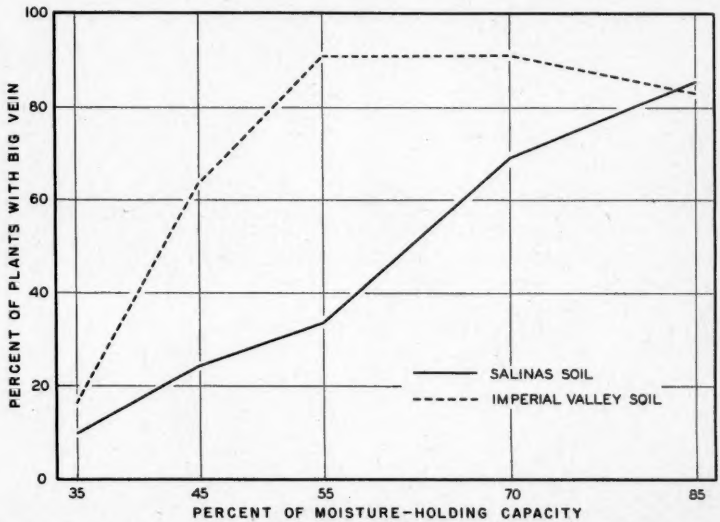

FIGURE 2.—The percentage of plants with big vein occurring on soils from Salinas and the Imperial Valley, held at different levels of moisture. Each point on the curve for the Salinas soil was based on 120 plants; for the Imperial Valley soil, on 79 to 90 plants.

This discrepancy was probably due largely to the difference in rate of growth but also perhaps to inherent differences between the soils. In general lettuce grows as well in the Imperial Valley as in the Salinas area. The former soil, however, has a rather high salt content and, when it was used in pots without drainage as in these experiments, the salinity built up to a point where the plants did not develop so rapidly as they did in the Salinas soil. Other explanations of the decreased fertility under conditions of poor drainage might be made, but at present this one seems the most logical. At any rate, since the plants in Imperial Valley soil grew more slowly, it was possible to continue the moisture treatments for a longer period than was feasible with the plants in the Salinas soil.

[12] Part of the data for the Imperial Valley soil were taken from the notes of the late I. C. Jagger.

Figure 3 indicates that big vein developed at about the same rate in both soils. However, almost all the big vein appearing on the plants after about 50 days from transplanting was in the series at 45 and 55 percent of the moisture-holding capacity. From this observation it appears that, if it had been possible to prolong the trials with Salinas soil, it is probable that the discrepancy between the two curves in figure 2 would have been reduced considerably.

The data of the foregoing discussion were based on plants grown from the one- or two-leaf stage to just before the heads began to form. Heading or seeding plants might conceivably present a somewhat different picture because their response to the environment is probably not the same as that of younger plants. Since field observations

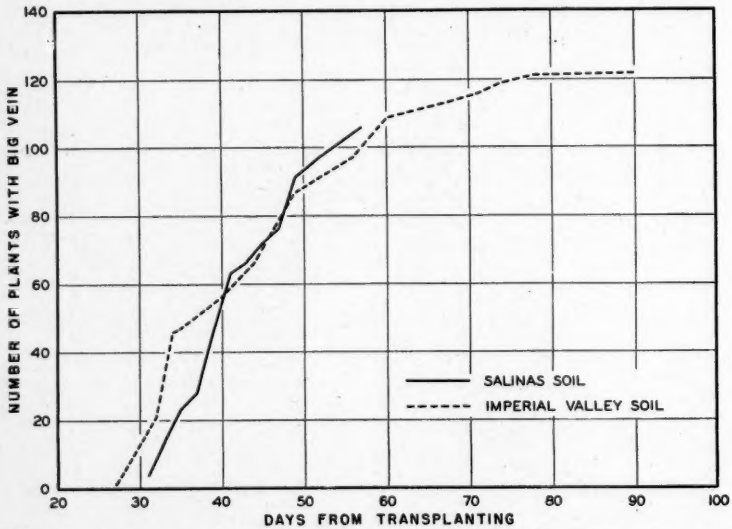

FIGURE 3.—Number of diseased plants in relation to number of days after transplanting as shown by a representative experiment in which 200 plants were grown on soil from Salinas and a similar experiment in which 200 plants were grown on soil from the Imperial Valley.

indicate that big vein is more likely to cause severe damage when plants are affected while young, a study of this early stage is of the most importance.

EFFECT OF SOIL MOISTURE AND BIG VEIN ON GROWTH

In table 1 are expressed the growth results from one representative experiment on each of the two soils studied. Figure 1, *B*, shows the comparative size of such plants. As would be expected, the different amounts of soil moisture had considerable effect on plant weight per pot. In all the experiments the largest yield was always obtained at the highest moisture level, although this level was probably somewhat above the optimum for field-grown lettuce. Since no allowance was

made for increase in plant weight as the experiment progressed, 85 percent of the moisture-holding capacity was above the optimum when the trial was started; but, as the plants grew, correspondingly less water was added when the pots were brought up to weight. This observation is substantiated by the fact that in the early part of the experiment the plants in the 70-percent series grew most rapidly, but after about a month they were overtaken by the plants at the higher evel.

TABLE 1.—*Average yield of lettuce per 4-plant pot at different approximate soil moisture levels*

Soil	Yield at indicated percent of the moisture-holding capacity				
	35	45	55	70	85
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
Salinas [1]	9.31	25.18	55.71	107.18	140.74
Imperial Valley [2]	2.60	25.24	68.74	79.04	85.85

[1] Minimum significant difference between moisture levels at 5-percent point=16.60; at 1-percent=22.27.
[2] Minimum significant difference between moisture levels at 5-percent point=13.45; at 1-percent=18.04.

Table 2 shows the average weight of diseased and healthy plants grown at different moisture levels. These data are from one experiment with each of the two soils used and are more or less typical of the others. The difference between the weights of the two classes of plants was in general not significant in most experiments, but the trend was the same. It will be noticed that diseased plants grown in Salinas soil tended to be larger than the healthy ones, while the opposite was true in the Imperial Valley soil. As in the discussion on symptoms, this discrepancy can be explained on a time basis.

At the end of two of the trials with Salinas soil the plant weights were tabulated in the order in which big vein developed on the plants in a given pot. Since the data from both trials were similar, the results from only one trial are shown in table 3. These figures are based on plants in the series at 70 and 85 percent of the moisture-holding capacity, where four diseased plants per pot was not an uncommon occurrence.

TABLE 2.—*Average weight of individual diseased and healthy plants at different soil moisture levels*

Soil	Plant condition	Weight [1] at indicated percent of the moisture-holding capacity				
		35	45	55	70	85
		Grams	*Grams*	*Grams*	*Grams*	*Grams*
Salinas	Healthy	2.12**	4.82**	12.22*	17.60*	26.26
	Diseased	3.80	8.51	16.24	28.75	36.46
Imperial Valley	Healthy	.66	6.69	20.71**	26.25**	29.45**
	Diseased	.61	5.93	16.30	18.14	17.14

[1] *=difference between healthy and diseased plants significant at the 5-percent level; **=significant at the 1-percent level.

TABLE 3.—*Length of disease period in relation to weight of plants grown on Salinas soil*

Number of diseased plants	Average disease period	Average weight
	Days	*Grams*
17	23.2	16.0
16	17.4	20.2
12	13.6	24.8
5	11.0	14.6

Except for the last plant diseased, it is evident that the longer a plant had big vein the less its weight at harvest. In all the experiments the fourth plant to become diseased in a pot was smaller than those previously affected. There appear to be two explanations for this: (1) The rosette plants mentioned previously were much stunted, and when they became affected with big vein, it was always near the end of an experiment; (2) throughout the experiments the more vigorously growing plants developed big vein most quickly. As a result, the smallest plants were the last to become diseased, which also tended to bring the average weight of the last group down. An observation which lends some support to this explanation is the fact that when three plants in a pot had big vein, the remaining healthy plant was generally smaller than at least two of the diseased plants.

DISCUSSION

The method of adding water to the soil in pots undoubtedly would cause moisture to be present in larger amounts near the central slotted pipes, particularly in the lower moisture series. As a result, root growth at the lower levels of moisture would be more or less restricted to the center of the pot. It is possible that the small root area resulting from a limited water supply was a more important factor in reducing the amount of big vein than the direct effect of decreased moisture itself. This would be particularly true if the disease were not distributed uniformly through the soil. However, since the nature of the disease-causing agent has not been demonstrated, it is not certain whether the results obtained were due to the effect of moisture upon the host, upon the causal agent, or upon the disease complex.

The results of the present investigations show that in trials of lettuce strains for resistance to big vein it is important to maintain soil moisture at a high level not only because big vein develops in a larger number of plants when abundant water is supplied but also because the plants become affected more quickly, thus enabling the investigator to eliminate susceptible individuals early in the trials.

Observations also indicate that if soil moisture is maintained at the best level for the growth of lettuce the disease also develops well. Therefore, control of moisture in the field offers little hope of reducing big vein under commercial lettuce-growing conditions.

SUMMARY

An investigation of soil moisture in relation to the big vein disease of lettuce was made on two different soils where big vein occurred, one from near Salinas, Calif., and the other from the Imperial Valley, Calif.

The number of plants that developed big vein on these soils increased with increasing moisture. A considerable number of diseased plants were present even at moisture levels below the optimum for growth of lettuce, and a few plants had big vein when only enough water was supplied to keep them alive. The highest incidence of disease occurred in the treatments that produced the largest plants.

Plant weight increased with each increment of moisture used. The more vigorously growing individuals in each series seemed to be most readily affected with big vein, but the earlier a plant showed the disease the smaller was its final weight. With the Salinas soil, diseased plants seemed to be larger than healthy plants in the same pot, while the reverse was true with the Imperial Valley soil. The difference was attributed to a longer growing period for plants in the Imperial Valley soil.

From the results obtained it is recommended that tests of lettuce for resistance to big vein be carried out on well-watered soil. Little hope is held for reducing the number of big vein plants in commercial fields through the control of soil moisture.

APPARENT PHOTOSYNTHESIS AND TRANSPIRATION OF PECAN LEAVES TREATED WITH BORDEAUX MIXTURE AND LEAD ARSENATE [1]

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INTRODUCTION

Two of the most widely used spray materials in the control of insects and diseases of the pecan are lead arsenate and bordeaux mixture. Since it has been clearly demonstrated (*6*, *8*, *12*) [2] that certain sprays may have a deleterious effect on the foliage of trees, it seemed advisable to determine to what extent bordeaux mixture and lead arsenate influence the photosynthesis and the transpiration of pecan leaves. Most of the literature relating to the effects of bordeaux mixture on these physiological processes is contradictory and was obtained with species other than the pecan. Lutz and Hardy (*10*) reported that pecan leaves sprayed with low-lime bordeaux mixture were more efficient in carrying on photosynthesis than unsprayed leaves. Clore (*2*), Hoffman (*8*), and others found no significant difference between the carbon dioxide intakes of bordeaux-sprayed and untreated apple leaves, whereas Southwick and Childers (*12*) found that bordeaux mixture sprayed on apple leaves caused a reduction in apparent photosynthesis. Some workers (*3*, *13*) reported an increase in the rate of transpiration due to the application of bordeaux mixture, while others (*1*, *4*, *11*) failed to find very marked effects of this spray material on transpiration rates. Few or no data are available relating to the effects of lead arsenate on carbon dioxide assimilation or transpiration.

In this paper are presented data which show the effects of bordeaux mixture and lead arsenate applications on the rates of apparent photosynthesis and transpiration of mature leaves of the pecan, *Carya illinoensis* K. Koch (syn. *C. pecan*), at Brownwood, Tex.

MATERIALS AND METHODS

The apparatus and procedure for measuring the apparent photosynthesis were similar to those used by Heinicke and Hoffman (*7*). The apparatus consisted of six carbon dioxide absorption towers, of which two were attached to the check leaves, two were attached to the test leaves, and two served as air controls. The assimilation chambers were similar to those described by Heinicke (*5*) and were adapted for use with pecan leaves by Loustalot and Hamilton (*9*). A continuous stream of air at the rate of 2 to 2.5 liters per square centimeter of leaf area per hour (*7*) was drawn through the chambers

[1] Received for publication September 14, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 19.

by mercury pumps. The water transpired by the leaves was determined by weighing, after it was absorbed by a dehydrating agent (pumice stone impregnated with sulfuric acid) through which the air was passed before it reached the carbon dioxide absorption towers. The volume of air passing through each absorption tower was accurately measured by a wet-test flowmeter.

All the experiments were conducted during the summer of 1941 with the mature leaves of a 10-year-old pecan tree of the Western variety growing near the laboratory of the United States Department of Agriculture Pecan Field Station, Brownwood, Tex. The tree was in good vigor and was carrying a full crop of nuts. The foliage, which was abundant and dark green, had been sprayed in late spring with zinc sulfate (3–100) to control pecan rosette, but at the time the experiments were started there was no visible residue.

Two pairs of mature, healthy, dark-green leaflets, comparable in position and exposure to sunlight, were selected for each experiment. One pair was designated as check and the other as test leaves before any determinations of transpiration or photosynthesis were begun. Two determinations were made each day in all the experiments—one between 9:00 a. m. and 1:15 p. m. and the other between 1:30 and 4:30 p. m. Weather observations, including temperature (°F.) and relative humidity (percent), were made at the beginning, the middle, and the end of each determination. Owing to lack of facilities for recording solar radiation, the general sky condition prevailing during each determination was estimated.

Apparent photosynthesis was calculated as the average number of milligrams of carbon dioxide assimilated per hour by 100 cm.^{2} of leaf surface. Transpiration was calculated as the average number of grams of water lost per hour by 100 cm.^{2} of leaf area. The relation in the apparent photosynthesis and transpiration was first established between the check and the test leaflets for a period of 4 to 5 days, after which the spray material was applied to the test leaflets.

Three experiments with bordeaux mixture were performed between June 27 and July 28, and two experiments with lead arsenate were conducted between September 2 and October 10. In all instances the spray material was applied to both surfaces of the test leaflets by dipping them twice into a thoroughly agitated, freshly prepared mixture and allowing the material to dry on the leaflets between immersions. The spray material was applied in the morning and had thoroughly dried on the leaves before the determinations were made.

It will be noted that in each of the five experiments the test leaves had lower rates of transpiration and apparent photosynthesis than the check leaves. This was purely accidental and has not particular significance. In setting up each experiment the two pairs of leaflets were selected, numbered, and designated test and check before any determinations were made.

EXPERIMENTS WITH BORDEAUX MIXTURE

The data for the three experiments with bordeaux mixture are presented graphically in figures 1 to 3. Since the relation between the two sets of leaflets before the spray application was fairly constant,

as shown in the figures, it was possible to obtain a good estimate of the efficiency of each set of leaves; any appreciable deviation from this established relation after the spray material was applied could be attributed to the treatment.

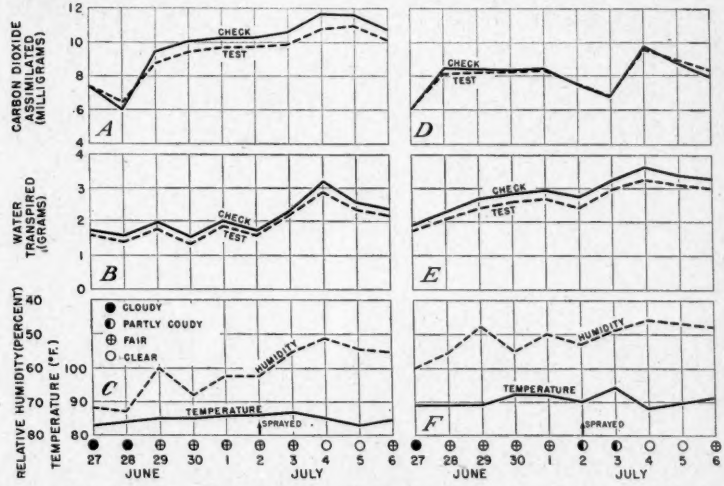

FIGURE 1.—The effects of a single application of bordeaux mixture on the apparent photosynthesis and transpiration of mature pecan leaves exposed to full sunlight: *A–C*, Morning determinations of carbon dioxide assimilated (*A*), of water transpired (*B*), and of the accompanying weather conditions (*C*); *D–F*, afternoon determinations of carbon dioxide assimilated (*D*), of water transpired (*E*), and of the accompanying weather conditions (*F*).

In experiment 1 (fig.1) both sets of leaflets were exposed to full sunlight during both the morning and the afternoon period except on cloudy days, when the light varied with the extent of cloudiness. Bordeaux mixture (6–2–100) was applied to the test leaflets at 8 a. m. on July 2. In the succeeding 5 days, inclusive of the day of spraying, the relation in the transpiration and the apparent photosynthetic activity between the two sets of leaflets differed very little from that established during the standardization period from June 27 to July 1. A maximum average temperature of 94° F. occurred on the second afternoon following the spray application but caused no noticeable effect on the transpiration and photosynthetic relations between the check and test leaflets. The variations in the apparent photosynthetic relation between the two pairs of leaflets on June 27 and 28 may have been due to some unbalanced internal conditions in the leaflets or to errors in experimental manipulation or determinations.

In experiment 2 (fig. 2) the procedure was similar to that in experiment 1, except that both sets of leaflets were partially shaded by adjacent foliage during both the morning and the afternoon period. Bordeaux mixture (6–2–100) was applied to the test leaflets on the morning of July 12. During the next 4 days, including the day the spray was applied, there was relatively little change in the relation in the two processes between the two sets of leaflets as compared with

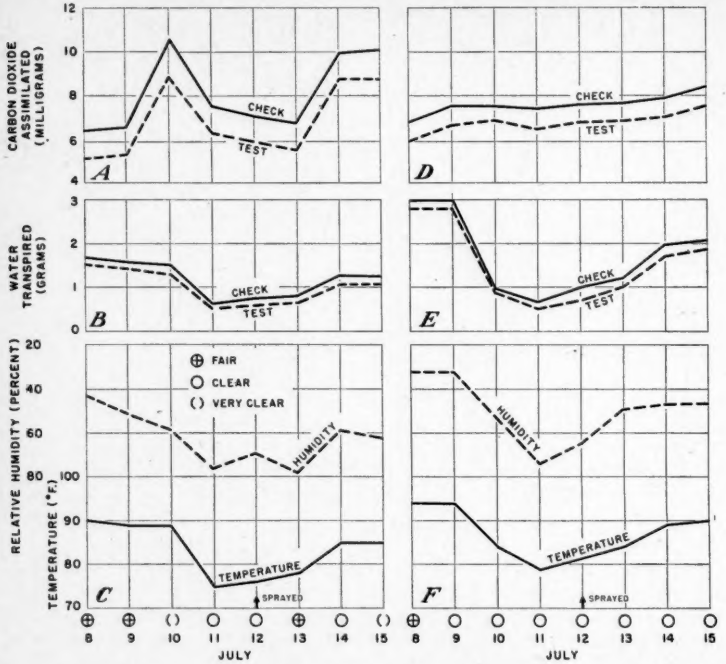

FIGURE 2.—The effects of a single application of bordeaux mixture on the apparent photosynthesis and transpiration of mature pecan leaves exposed to partial sunlight: *A–C*, Morning determinations of carbon dioxide assimilated (*A*), of water transpired (*B*), and of the accompanying weather conditions (*C*); *D–F*, afternoon determinations of carbon dioxide assimilated (*D*), of water transpired (*E*), and of the accompanying weather conditions (*F*).

that during the standardization period. The temperature during this period was unusually low for the time of year.

In experiment 3 (fig. 3) the exposure of both sets of leaflets during the morning and the afternoon period was similar to that described for the leaflets used in experiment 1. This experiment differed from experiments 1 and 2 in that the test leaflets received three applications of bordeaux mixture, each on a different day, and the third application was of an 8–8–100 mixture instead of the recommended 6–2–100. The first application was made on July 20, the second on July 23, and the third on July 25. The test leaflets were so well covered with spray residue at the end of the third application that the green color of the leaflets was hardly visible. The results were more or less similar to those obtained in experiments 1 and 2—that is, the relation in the two processes between sprayed and check leaflets remained fairly constant both before and after the application of bordeaux mixture to test leaflets. The fact that the apparent photosynthesis was not affected in the leaflets covered by three spray applications indicates that the absorption of light energy was not sufficiently reduced by the spray residue to influence the rate of carbon dioxide assimilation.

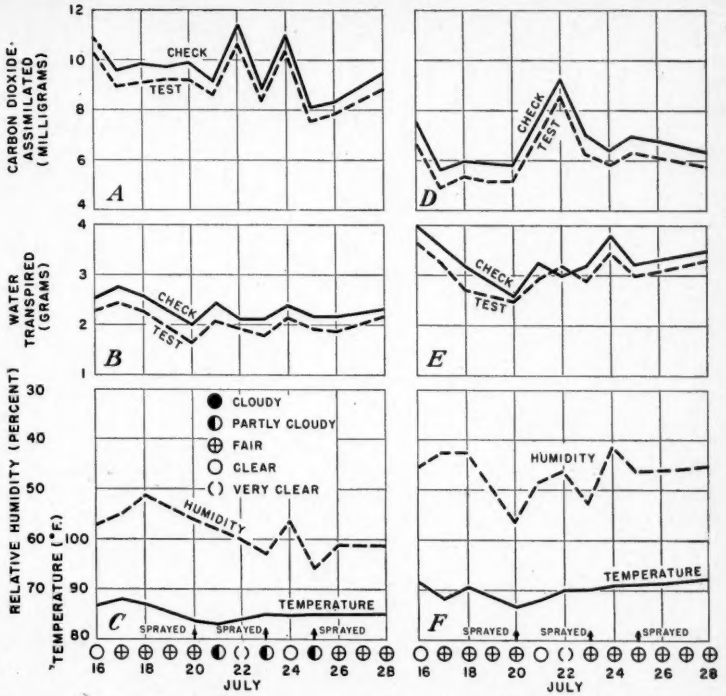

FIGURE 3.—The effects of three successive applications of bordeaux mixture on the apparent photosynthesis and transpiration of mature pecan leaves exposed to full sunlight: *A–C*, Morning determinations of carbon dioxide assimilated (*A*), of water transpired (*B*), and of the accompanying weather conditions (*C*); *D–F*, afternoon determinations of carbon dioxide assimilated (*D*), of water transpired (*E*), and of the accompanying weather conditions (*F*).

The data show that under the conditions of these experiments bordeaux mixture had little or no effect on the apparent photosynthesis and transpiration of mature pecan leaves. It will be noted from the photosynthesis and transpiration curves that small but inconsistent deviations occurred in the relation between the check and test leaflets, but these deviations fall within the limits of experimental error since they are no greater than differences between duplicate determinations from a single pair of leaflets. The deviations in duplicate determinations were usually less than 5 percent, but in a few instances they were higher, the maximum being about 14 percent. The variations in the ratios between the test and check leaflets, before and after the spray application, were usually less than 5 percent, the maximum being approximately 7 percent.

Lutz and Hardy (*10*) reported a higher photosynthetic rate for pecan leaves sprayed with low-lime bordeaux than for unsprayed leaves. However, they pointed out that the unsprayed leaves were lighter in color than those sprayed and in addition contained diseased areas. In the same paper these authors presented data indicating

that dark-green pecan leaves are more efficient in carbon dioxide assimilation than those of a lighter green color, a fact that Heinicke (*6*) demonstrated with apple leaves. It has also been shown (*9*) that even a mild infection by the fungus causing the disease known as downy spot [*Mycosphaerella caryigena* (Demaree and Cole)] markedly reduces the ability of pecan leaves to assimilate carbon dioxide. In view of these facts, it seems reasonable to assume that the beneficial effects of bordeaux mixture on the efficiency of pecan leaves observed by Lutz and Hardy may be attributed to the indirect effect on disease and the intensification of green color rather than to the direct effect of the spray material itself. It should be emphasized in this connection that the leaves used in the experiments reported herein were dark green and free from any visible disease injury, and that, therefore, any direct influence of bordeaux mixture on the two physiological processes could be determined.

EXPERIMENTS WITH LEAD ARSENATE

In the experiments with lead arsenate the same general procedure was followed as in the bordeaux mixture experiments. The first experiment was begun on September 2 and terminated on September 13, and the exposure of the two pairs of leaflets for morning and afternoon periods was similar to that described for the leaflets used in experiment 1 with bordeaux mixture. Lead arsenate (6–100) was applied to the test leaflets on three different dates, September 8, 10, and 12.

In the second experiment, started on September 29 and terminated on October 10, one spray application was made on the morning of October 6. In this case the two pairs of leaflets were located on the east side of the tree and exposed in such a way as to receive full benefit from sunlight, except when cloudy weather obscured the sun, but they were fully shaded by the adjacent foliage during the afternoon periods. No determinations were made on the afternoons of October 4 and 5.

The experimental data for the lead arsenate experiments are presented in figures 4 and 5. These data show no appreciable effect of lead arsenate applications on the rates of transpiration and carbon dioxide assimilation of pecan leaves. There was a wide variation in the average temperature during the experimental periods from September 8 to 13 (fig. 4), the maximum of 93° F. occurring in the afternoon period of September 8 and the minimum of 67° occurring during the morning period of September 9. The average relative humidity varied from a low of 34 percent during the afternoon of September 8 to a high of 96 percent during the morning of September 9. With decreases in temperature and increases in relative humidity the rate of transpiration decreased, but in neither experiment (figs. 4 and 5) was there any appreciable difference in the established ratio between the check and test leaflets. Apparent photosynthesis varied considerably with fluctuations in light intensity and other meteorological factors, but in no instance was there any marked change in the established relation in this process between sprayed and unsprayed leaflets. The minor deviations that occurred in the ratio of both transpiration and apparent photosynthesis rates in test and check leaflets are within the limits of experimental error. Therefore, appli-

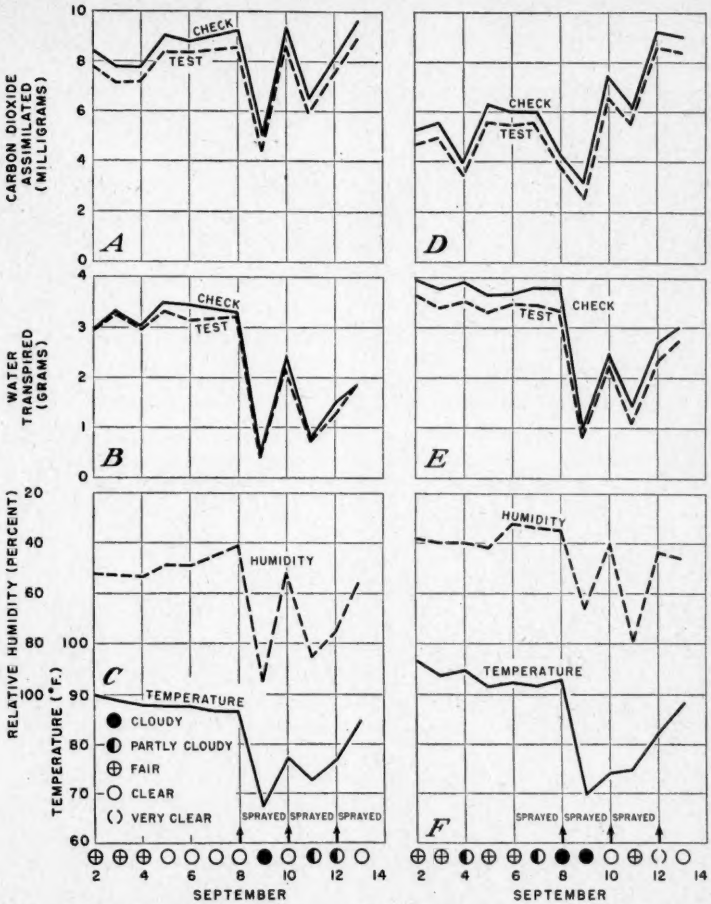

FIGURE 4.—The effects of three successive applications of lead arsenate on the apparent photosynthesis and transpiration of mature pecan leaves exposed to full sunlight: *A–C*, Morning determinations of carbon dioxide assimilated (*A*), of water transpired (*B*), and of the accompanying weather conditions (*C*); D–F, afternoon determinations of carbon dioxide assimilated (*D*), of water transpired (*E*), and of the accompanying weather conditions (*F*).

cations of lead arsenate to mature pecan leaves had no significant effect on carbon dioxide assimilation or transpiration.

The fluctuations in the apparent photosynthesis from day to day of both test and check leaflets apparently were due to changes in environmental conditions, especially light, while fluctuations in transpiration seemed to follow changes in temperature and relative humidity.

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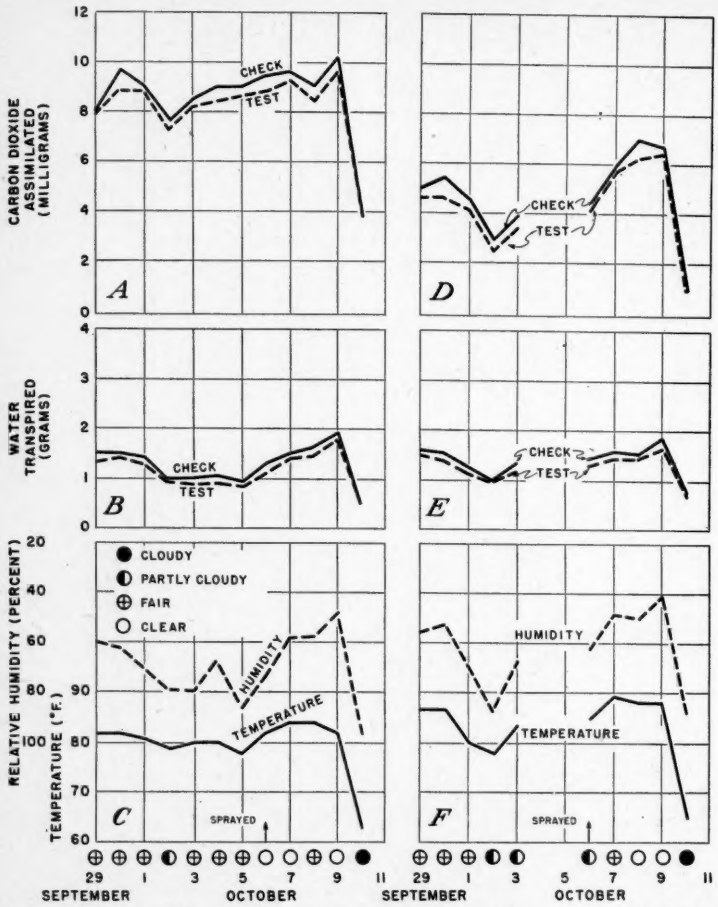

FIGURE 5.—The effects of a single application of lead arsenate on the apparent photosynthesis and transpiration of mature pecan leaves exposed to full sunlight in the morning and full shade in the afternoon: *A–C*, Morning determinations of carbon dioxide assimilated (*A*), of water transpired (*B*), and of the accompanying weather conditions (*C*); *D–F*, afternoon determinations of carbon dioxide assimilated (*D*), of water transpired (*E*), and of the accompanying weather conditions (*F*).

SUMMARY

Studies were made to determine the effects of bordeaux mixture and lead arsenate applications on the rates of the apparent photosynthesis and transpiration of mature pecan leaves on a 10-year-old pecan tree during the 1941 season. Three experiments were conducted with bordeaux mixture during the period from June 27 to July 28, and two similar experiments with lead arsenate were carried on between September 2 and October 10.

No appreciable effects on the apparent photosynthesis or transpiration were obtained by applications of either bordeaux mixture or lead arsenate to mature pecan leaves. The same results were obtained from one or three applications of the spray materials even though the leaves receiving three applications were so well covered that the green color was scarcely visible.

Wide fluctuations occurred in rates of apparent photosynthesis and transpiration from day to day and during the morning and afternoon periods of the same day, but apparently these were due largely to changes in meteorological conditions. The rates of apparent photosynthesis were affected primarily by fluctuations in light intensity, while transpiration rates seemed to follow changes in temperature and relative humidity.

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CYTOLOGICAL AND GENETIC ANALYSIS OF CHROMOSOMAL ASSOCIATION AND BEHAVIOR DURING MEIOSIS IN HEXAPLOID TIMOTHY (PHLEUM PRATENSE)[1]

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INTRODUCTION

A knowledge of the type of chromosomal association occurring during meiosis is important not only for an understanding of the origin of the species but also for the intelligent planning of a breeding program. In American timothy (*Phleum pratense* L.), a hexaploid with $2n=42$, evidence regarding the kind of chromosome pairing is contradictory. The experiments reported in this paper were designed to furnish further information on this subject.

REVIEW OF LITERATURE

Gregor (*5*)[2] and Gregor and Sansome (*6*) reported synthesis of a hexaploid type by spontaneous doubling in the hybrid *Phleum pratense* ($2n=14$) $\times$ *P. alpinum* ($2n=28$), which was cross-fertile with ordinary hexaploid *P. pratense*. On the basis of these results and the behavior in a natural hybrid which he supposed to be *P. pratense* ($2n=14$) $\times$ *P. pratense* ($2n=42$), Müntzing (*8*) concluded that American timothy was an allohexaploid. Later, however, Nordenskiöld (*16*) reported 14 bivalents in F_1 *P. pratense* ($2n=14$) $\times$ *P. pratense* ($2n=42$), Müntzing and Prakken (*10*) found 28 bivalents in 63-chromosome timothy plants obtained from twin seedlings, and Myers (*12*) observed a maximum of 12 bivalents in F_1 plants of *P. pratense* ($2n=42$) $\times$ *P. subulatum* ($2n=14$). In polyhaploid ($2n=21$) plants obtained from twin seedlings of hexaploid timothy, Nordenskiöld (*17*) found 7 bivalents plus 7 univalents. These results were in agreement with the hypothesis that at least 2 of the 3 genomes of the reduced complement in timothy were homologous. On the other hand, Müntzing (*8*) and Nordenskiöld (*16*) stated that multivalents were not found in meiosis in the hexaploid, while Müntzing and Prakken (*10*) reported that multivalents were very rare, if they occurred at all. Myers (*12*) found a low frequency of quadrivalents in the 1 plant studied. The apparently contradictory evidence of homology between genomes and absence of multivalents was discussed in detail by Müntzing and Prakken (*10*), and they raised the question whether each of the bivalents was formed always from the same 2 chromosomes or whether 2 or 3 bivalents were formed by random pairing of 4 or 6 homologues.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 32.

There is a paucity of critical genetic evidence from hexaploid timothy. Barker and Hayes (*1*), Proytchoff, according to Horsfall (*7*), and Myers and Chilton [3] obtained typical monohybrid ratios for resistance to rust. Clarke (*3*) explained the occurrence of chlorophyll-deficient seedlings among inbred progenies by the assumption of triplicate factors. Using similar material, Wexelsen (*20*) obtained results that he interpreted on the basis of single, duplicate, and triplicate factors, assuming that the species was an allohexaploid. In two families, however, he obtained results that suggested the possibility of tetrasomic inheritance.

MATERIAL AND METHODS

For the cytological studies, plants were selected from different sources as follows: (1) Three plants from three seed collections in New Jersey, (2) one plant from Cornell strain No. 1777, (3) three F_1 hybrids involving parents from seed collected in New Hampshire and Vermont, and (4) three plants from open-pollinated seed produced on a plant selection from S48, pasture-hay strain from the Welsh Plant Breeding Station, Aberystwyth, Wales.

The plants were grown in the greenhouse during the winter of 1940–41, and microsporocyte material was killed and fixed in acetic alcohol (1 part glacial acetic acid to 3 parts absolute alcohol). All data were collected and the photomicrographs made from fresh acetocarmine smear slides.

For the genetic studies, five unrelated plants were selected that were known from previous tests to be heterozygous for a factor or factors conditioning albino seedlings. Selfed seed from these plants and from their first inbred-generation (I^1) progenies were germinated in Petri dishes, and the seedlings were classified for green versus albino.

EXPERIMENTAL RESULTS

CYTOLOGICAL INVESTIGATIONS

Diakinesis

The type of chromosomal association was determined at diakinesis for 7 of the plants (table 1). Of the 102 sporocytes examined, only 2 had 21 bivalents; all the others had quadrivalents, sexivalents, or both, in addition to bivalents. The frequency of quadrivalents in the sprocytes ranged from 1 to 7, the average being 2.9 to 4.9 quadrivalents per sporocyte among the 7 plants. It is worthy of note that the range in quadrivalent frequency is similar to that found among plants of *Dactylis glomerata* L. (*14*, *15*) and of tetraploid *Lolium perenne* L. in which there are 4 genomes of 7 chromosomes each.[4] Sexivalents were found in 33 of the 102 sporocytes; of these, 26 had 1 sexivalent (fig. 1, *A* and *B*), 6 had 2, and 1 had 3. In 1 of the sporocytes with a sexivalent the nucleolar chromosomes apparently were involved (fig. 1, *B*), whereas in the sporocyte with 3 sexivalents, none consisted of nucleolar chromosomes. The results indicate that at least 4 of the 7 chromosomes are capable of pairing with their homologous or homeologous chromosomes from the other 5 genomes. Sexivalents were found in 1 or more

[3] Myers, W. M., and Chilton, S. J. P. [Unpublished data obtained at the U. S. Regional Pasture Research Laboratory.]

[4] Myers, W. M. [Unpublished data obtained at the U. S. Regional Pasture Research Laboratory.]

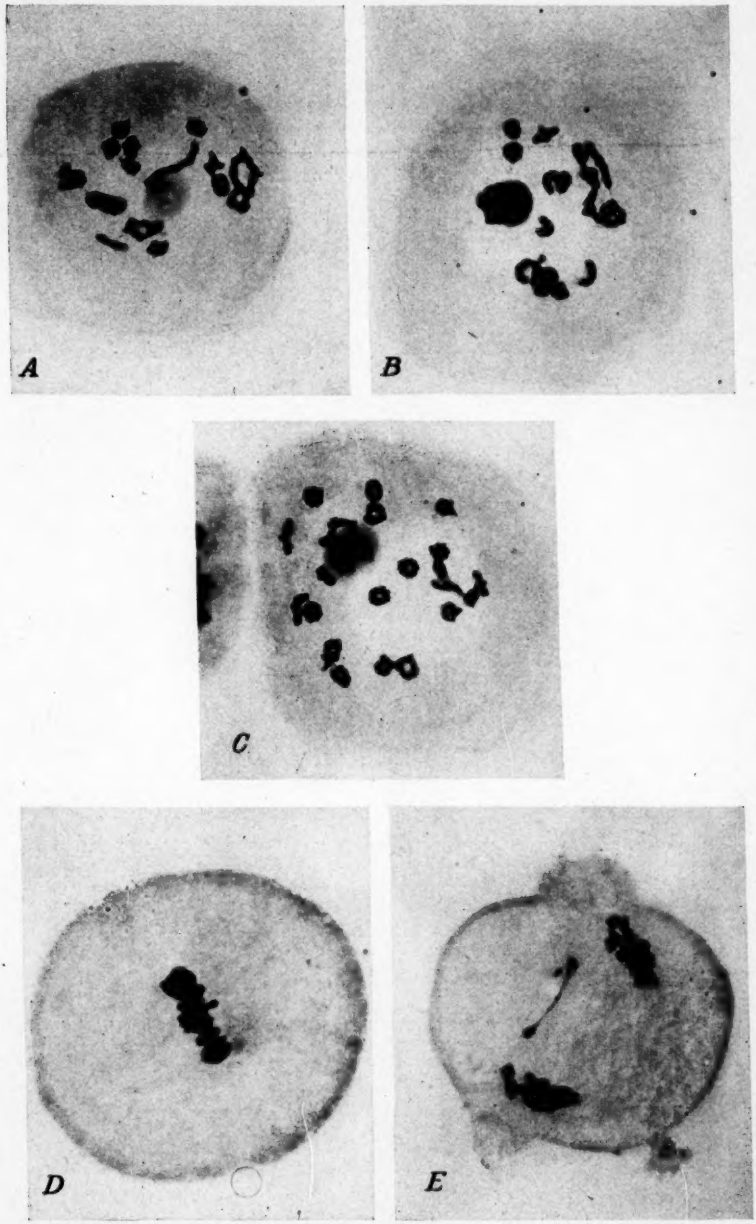

FIGURE 1.—*A*, Diakinesis in *Phleum pratense* with one sexivalent plus quadrivalents and bivalents; *B*, diakinesis with a sexivalent lying on the nucleolus; *C*, diakinesis showing secondary association of the bivalents; *D*, metaphase I with ring quadrivalent; *E*, late anaphase I with dicentric chromatid bridge and acentric fragment.

sporocytes in 6 plants, while in the other plant only 9 sporocytes were examined.

TABLE 1.—*Frequency of bivalents, quadrivalents, sexivalents, and half-chiasmata per chromosome at diakinesis in 7 plants of Phleum pratense*

Chromosome groups per cell or half-chiasmata per chromosome (number)	Cells, chromosomes, or half-chiasmata in plant No.—							Total cells
	Ti 49 (4)	Pot 1441	Pot 1443	Ti 11 (8)	Ti 14 (5)	Ti 16 (5)	Ti 46 (9)	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Chromosomes per cell:								
2_{II}	1	0	0	0	1	0	0	2
$1_{II}+1_{IV}$	3	1	1	0	0	0	0	5
$1_{II}+2_{IV}$	5	1	1	0	3	0	0	10
$1_{II}+3_{IV}$	8	4	1	2	2	1	0	18
$1_{II}+4_{IV}$	5	3	1	2	3	1	0	15
$1_{II}+5_{IV}$	2	0	1	0	1	4	0	8
$1_{II}+6_{IV}$	1	0	2	0	2	1	4	10
$1_{II}+7_{IV}$	0	0	0	0	0	1	0	1
$1_{II}+1_{IV}+1_{VI}$	1	0	1	0	0	0	0	2
$1_{II}+2_{IV}+1_{VI}$	3	0	0	1	1	0	1	6
$1_{II}+3_{IV}+1_{VI}$	4	0	1	0	0	0	1	6
$1_{II}+4_{IV}+1_{VI}$	1	0	2	0	0	0	1	4
$1_{II}+5_{IV}+1_{VI}$	2	0	0	0	0	2	1	5
$1_{II}+6_{IV}+1_{VI}$	0	0	0	0	0	0	3	3
$1_{II}+0_{IV}+2_{VI}$	0	0	1	0	0	0	0	1
$1_{II}+2_{IV}+2_{VI}$	0	0	1	0	0	0	0	1
$1_{II}+3_{IV}+2_{VI}$	0	0	0	0	0	1	1	2
$1_{II}+4_{IV}+2_{VI}$	0	0	0	0	0	0	1	1
$5_{II}+5_{IV}+2_{VI}$	0	0	0	0	0	0	1	1
$8_{II}+2_{IV}+3_{VI}$	0	0	1	0	0	0	0	1
Total cells	36	9	14	5	13	11	14	102
Average bivalents per cell	14.2	15.0	12.5	14.0	14.2	10.3	8.5	.....
Average quadrivalents per cell	2.9	3.0	3.1	3.2	3.3	4.8	4.9	.....
Average sexivalents per cell	.3	0	.8	.2	.1	.4	.9	.....
Half-chiasmata per chromosome in—								
Bivalents	1.64	1.88	1.75	1.77	1.90	1.56	1.70	.....
Quadrivalents	1.70	1.94	1.85	1.91	1.86	1.82	1.79	.....
Sexivalents	1.88	.....	1.94	1.67	2.00	1.83	1.87	.....
Average half-chiasmata per chromosome	1.67	1.90	1.80	1.81	1.89	1.69	1.76	.....

In most sporocytes there was a tendency for the bivalents to lie together in pairs (fig. 1, *C*). In many cases it was impossible to determine whether a configuration consisted of four chromosomes associated as a quadrivalent or of two bivalents lying so close together that they touched, and such doubtful cases were recorded as two bivalents. Müntzing (*8*) observed cases of secondary association at metaphase I in *Phleum pratense*. It is the opinion of the author that the association of bivalents in pairs at diakinesis in this material was a relic of multivalent pairing at prophase rather than a secondary attraction of bivalents during diakinesis.

Among the seven plants, the average frequency of half-chiasmata per chromosome ranged from 1.67 to 1.90 (table 1). It is apparent from an examination of table 1 that chiasma frequency was not closely related to bivalent frequency among the different plants. If chiasma frequency were the principal factor conditioning variations among plants in the formation of multivalents, a negative correlation of chiasma with bivalent frequency would have been expected. Instead, a correlation coefficient of +0.437 was obtained. With

five degrees of freedom, this value is not significant statistically (*4, table VA*); nevertheless, the results suggest that factors other than chiasma frequency have played a major role in conditioning the differences among plants in the formation of multivalents.

Although other factors appear to have been more important than chiasma frequency in determining differences among plants, there is evidence, nevertheless, that chiasma frequency was a limiting factor in multivalent formation. Within plants, the number of half-chiasmata per chromosome was higher for the chromosomes associated as quadrivalents than for those associated as bivalents in all but one plant. Likewise, the chiasma frequency per chromosome in the sexivalents was higher than that in the quadrivalents in all but one plant. Upcott (*19*) has pointed out that two opposing forces are involved in the relationship of chiasma frequencies in bivalents and multivalents. In those cases where chiasma frequency is not a limiting factor in multivalent formation, the number of chiasmata per chromosome should be lower in multivalents than in bivalents because of the less complete pairing resulting from exchanges of partners in prophase. On the other hand, where chiasma frequency is limiting, the chromosomes associated as multivalents represent a selected group with a higher frequency of chiasmata per chromosome. The plants used in this study apparently fall in the latter category.

Metaphase I

With the technique used, metaphase I was unsuited for determination of bivalent and multivalent frequency. The chromosomes were contracted and clumped together on the equatorial plate. Nevertheless, quadrivalents could be distinguished with certainty in numerous sporocytes (fig. 1, *D*). Also, it was possible at this stage to determine the frequency of sporocytes with unpaired chromosomes. The average percentage of sporocytes with univalents ranged from 1.1 to 14.3 percent for the 10 plants (table 2).

Table 2.—*Percentages of metaphase I sporocytes with univalents, of anaphase I sporocytes with lagging and dividing univalents and with dicentric chromatid bridges and acentric fragments, and of quartets with micronuclei*

Plant No.	Metaphase I		Anaphase I			Quartets	
	Cells	Sporocytes with univalents	Cells	Sporocytes with—		Cells	With micronuclei
				Lagging univalents	Bridge+fragment		
	Number	*Percent*	*Number*	*Percent*	*Percent*	*Number*	*Percent*
Ti 46 (9)	89	1.1	21	0	4.8	114	3.5
Ti 14 (5)	194	4.1	35	0	0	110	0
Pot 1443	124	4.8	23	4.4	4.4	51	9.8
Ti 11 (8)	124	4.8	56	1.8	0	101	0
Ti 7 (9)	109	6.4	29	0	0	81	3.7
Ti 49 (4)	93	6.5	29	3.4	0	99	8.1
Ti 160 (1)	239	9.2	54	3.7	7.4	87	1.2
Pot 1439	52	13.5	75	6.7	5.0	70	8.6
Pot 1441	50	14.0	26	23.1	3.8	66	15.1
Ti 16 (5)	126	14.3	28	17.8	0	75	6.7

Anaphase I

In 3 plants, no lagging univalents were observed in the 21, 29, and 35 sporocytes examined. In the other 7 plants, 1 or more sporocytes had lagging univalents that were undergoing equational division, the frequency of such sporocytes ranging from 1.8 to 23.1 percent for the different plants (table 2).

In five of the plants, 3.8 to 7.4 percent of the anaphase I sporocytes had a dicentric chromatid bridge accompanied by an acentric fragment (fig. 1, *E*), indicating that these plants were heterozygous for a chromosomal aberration, probably an inversion. In the plants in which bridges and fragments were not observed, the numbers of sporocytes examined were not sufficient to preclude the possibility of their occurrence.

Quartets

The frequency of quartets with micronuclei ranged from 0 to 15.1 percent for the 10 plants and appeared to be correlated with the frequency of laggards at anaphase I (table 2).

GENETIC INVESTIGATIONS

Calculated Ratios

In a hexaploid species, such as *Phleum pratense*, 3 different types of chromosomal behavior may be obtained: (1) The species may be an allohexaploid, in which case 21 bivalents will be formed regularly; (2) the species may be an autohexaploid in which the homologous chromosomes of the 6 genomes pair at random to form bivalents, quadrivalents, and sexivalents; or (3) there may be 4 genomes of one kind and 2 of another, so that the chromosomes of the former will pair at random, forming bivalents and quadrivalents, while the chromosomes of the latter genomes regularly form bivalents. In addition, there may be any intermediate condition between these extremes, depending upon the degree of differentiation of the chromosomes of the different genomes.

On the first assumption, duplicate and triplicate factors segregating in monohybrid, dihybrid, and trihybrid ratios would be expected. If the second condition obtains, hexasomic ratios would result (table 3). In the third type, various combinations of tetrasomic and disomic (monohybrid) ratios would be obtained. The expected ratios on this hypothesis for all genotypes from which segregation would occur in I_1 or I_2 are given in table 4. In this case the gene *Aa* is assumed to be located in chromosomes of the four homologous genomes, while the gene *Bb*, a duplicate of *Aa*, is located in chromosomes of the remaining two genomes. There is also the possibility that no duplicate of *Aa* occurred in the remaining two genomes, in which case only tetrasomic ratios would be expected. In plants in which multivalent pairing occurs, the genetic ratios are conditioned by position of the gene with reference to the centromere as well as by the relative number of dominant and recessive alleles that are present. The effects of the position of the gene have been illustrated in detail in an earlier paper (*13*), and these effects have been taken into account in the calculation of the ratios presented in tables 3 and 4.

TABLE 3.—*Calculated ratios in I_1 and I_2 from parents of different genotypes on the assumption of random pairing of the homologous chromosomes from 6 genomes*

ON THE ASSUMPTION OF CHROMOSOME ASSORTMENT

Genotype of parent	Ratio in I_1	Number of dominant I_1 phenotypes showing indicated segregation in I_2			
		∞:0	399:1	24:1	3:1
A_6	Not segregating	All	0	0	0
A_5a_1	do	All	0	0	0
A_4a_2	do	18.0	6.0	1.0	0
A_3a_3	399:1	6.6	9.1	5.6	1.0
A_2a_4	24:1	1.0	6.0	11.0	6.0
A_1a_5	3:1	0	0	1.0	2.0

ON THE ASSUMPTION OF CHROMATID ASSORTMENT

Genotype of parent	Ratio in I_1	Number of dominant I_1 phenotypes showing indicated segregation in I_2				
		∞:0	3,024:1	120:1	14.4:1	2.4:1
A_6	Not segregating	All	0	0	0	0
A_5a_1	do	360.0	105.0	18.0	1.0	0
A_4a_2	3,024:1	40.8	46.7	29.2	8.3	1.0
A_3a_3	120:1	1.1	3.2	4.7	3.2	1.0
A_2a_4	14.4:1	1.0	8.0	28.0	44.8	31.4
A_1a_5	2.4:1	0	1.0	18.0	105.0	216.0

TABLE 4.—*Calculated ratios in I_1 and I_2 from parents of different genotypes on the assumption of random association of homologous chromosomes of 4 genomes and pairing of homologous chromosomes of remaining 2 genomes*

ON THE ASSUMPTION OF CHROMOSOME ASSORTMENT

Genotype of parent	Ratio in I_1	Number of dominant I_1 phenotypes showing indicated segregation in I_2				
		∞:0	143:1	35:1	15:1	3:1
A_3a_1 *Bb*	Not segregating	13.0	2.0	1.0	0	0
A_3a_1 *bb*	do	3.0	0	1.0	0	0
A_2a_2 *Bb*	143:1	6.3	3.6	1.8	1.6	1.0
A_2a_2 *bb*	35:1	1.0	0	2.2	0	1.0
A_1a_3 *Bb*	15:1	4.0	2.0	1.0	4.0	4.0
A_1a_3 *bb*	3:1	0	0	1.0	0	2.0
a_4 *Bb*	3:1	1.0	0	0	0	2.0

ON THE ASSUMPTION OF CHROMATID ASSORTMENT

Genotype of parent	Ratio in I_1	Number of dominant I_1 phenotypes showing indicated segregation in I_2						
		∞:0	3,135:1	783:1	86.1:1	20.8:1	12.9:1	3:1	2.5:1
A_3a_1 *Bb*	3,135:1	729.5	360.0	180.0	174.0	87.0	24.0	1	12.0
A_3a_1 *bb*	783:1	9.4	0	15.0	0	7.2	0	0	1.0
A_2a_2 *Bb*	86.1:1	12.4	5.3	2.7	9.1	4.6	5.3	1	2.7
A_2a_2 *bb*	20.8:1	1.0	0	5.3	0	9.1	0	0	5.3
A_1a_3 *Bb*	12.9:1	32.8	2.0	1.0	14.5	7.2	30.0	18.8	15.0
A_1a_3 *bb*	2.5:1	1.0	0	24.0	0	174.0	0	0	360.0
a_4 *Bb*	3:1	1.0	0	0	0	0	0	2	0

Observed Ratios

In the I_1 generation of two of the plants, namely, Ti 2 (11) and Ti 6 (15), the ratios of green to albino seedlings were 546:19 and 626:24, respectively (table 5). As revealed by the χ^2 test, the deviations of these ratios from either 15:1 or 63:1 were too great to be attributed to the errors of random sampling. On the other hand, the fit to the calculated ratios 35:1, 20.8:1, and 24:1 was satisfactory. Therefore, the results in I_1 were in agreement with either of the following hypotheses: (1) That the plants were duplex for the gene located in the four homologous genomes, that the duplicate gene in the remaining two genomes was homozygous recessive or absent, and that either chromosome (35:1 ratio) or chromatid (20.8:1 ratio) assortment obtained (table 4), or (2) that the plants were duplex with six homologues pairing and disjoining at random (table 3).

In the I_2 generation of Ti 2 (11), 23 lines averaging 99.3 seedlings per line were classified (table 6). The character used in these investigations was lethal, and consequently all I_2 lines reported here and later in the paper were progenies of normal I_1 plants. Homozygous recessive I_2 lines were neither expected nor obtained. The fit of the I_2 lines to the calculated dihybrid ratio was on the border line of statistical significance (P lies between 0.02 and 0.05). When χ^2 was determined for each segregating family and the values summed, total χ^2 gave P of less than 0.01. The goodness of fit of observed to calculated trihybrid ratios (not shown in table 6) was less satisfactory than the fit to the dihybrid ratios. Therefore, the results in I_2 support the conclusion drawn from I_1 that dihybrid and trihybrid ratios were not obtained in the progeny of Ti 2 (11).

Table 5.—*Obtained ratios and goodness of fit to various calculated ratios in the first inbred generation of 5 plants of Phleum pratense*

Plant No.	Obtained ratio		Value of P of χ^2 for fit to indicated ratio							
	Green	Albino	143 : 1	120 : 1	86.1 : 1	63 : 1	35 : 1	24 : 1	20.8 : 1	15 : 1
Ti 2 (11)	546	19	<0.01	<0.01	<0.01	<0.01	0.30–0.50	0.30–0.50	0.10–0.20	<0.01
Ti 6 (15)	626	24	<.01	<.01	<.01	<.01	.10–.20	.70–.80	.20–.30	<.01
Ti 16 (5)	575	4	>.99	.70–0.80	.30–0.50	.05–0.10	<.01	<.01	<.01	<.01
Ti 15 (5)	632	0	.02–0.05	.02	<.01	<.01	<.01	<.01	<.01	<.01
Ti 11 (8)	557	0	.02–.05	.02–.05	.01–.02	<.01	<.01	<.01	<.01	<.01

On the basis of the hypothesis of duplex constitution of four homologous genomes with the duplicate gene in the remaining genomes homozygous recessive (A_2a_2bb), the fit of I_2 lines to calculated was good when either chromosomal or chromatid assortment was assumed. Furthermore, total χ^2 gave P of 0.10 to 0.20 on the hypothesis of chromosomal assortment, but P was less than 0.01 for fit of observed to the calculated for random chromatid segregation. The fit also was good of the observed ratio of I_2 lines to the expected ratio on the hypothesis that the parent plant was duplex with hexasomic chromosome pairing (A_2a_4). Total χ^2 gave P between 0.02 and 0.05 in this case.

TABLE 6.—*Obtained and calculated ratios and goodness of fit to various hypotheses in the second inbred generation of 4 plants of Phleum pratense*

Parent No.	Hypothetical genotype	Number of I_2 lines—			Value of P for—	
		Not segregating	Segregating		I_2 lines	Total χ^2 [3]
			35 : 1, 24 : 1, 20.8 : 1, or 15 : 1	3 : 1 or 2.5 : 1		
Ti 2 (11)		[1] 7.00	[1] 12.00	[1] 4.00		
	$A_1a_2\ bb$ [2]	5.88	11.77	5.35	0.70–0.80	0.10–0.20
	$A_2a_2\ bb$ [3]	7.00	10.11	5.89	.50–.70	<.01
	A_2a_4 [2]	6.72	10.56	5.76	.50–.70	.02–.05
	$Aa\ Bb$	10.71	6.12	6.12	.02–.05	<.01
Ti 6 (15)		[1] 3.00	[1] 11.00	[1] 8.00		
	$A_2a_2\ bb$ [2]	5.63	11.26	5.12	.20–.30	<.01
	$A_2a_2\ bb$ [3]	6.68	9.64	5.62	.10–.20	.30–.50
	A_2a_4 [2]	6.44	10.12	5.52	.20–.30	.10–.20
	$Aa\ Bb$	10.29	5.88	5.88	<.01	.02–.05
Ti 15 (5)		[1] 16.00	[1] 9.00			
	$A_1a_2\ bb$ [2]	18.75	6.25		.20–.30	.50–.70
	$A_1a_1\ bb$ [3]	19.01	5.18	.72	.10–.20	.05–.10
	$A_2a_2\ Bb$ [2]	17.30	5.94	1.75	.10–.20	(4)
	$A_2a_2\ Bb$ [3]	11.83	11.02	2.15	.10–.20	(5)
	A_1a_3 [2]	17.58	6.27	1.12	.20–.30	.10–.20
Ti 11 (8)		[1] 14.00	[1] 9.00			
	$A_1a_1\ bb$ [2]	17.25	5.75		.20–.30	.50–.70
	$A_1a_1\ bb$ [3]	17.20	5.08	.70	.10–.20	.02–.05
	$A_2a_2\ Bb$ [2]	16.34	4.95	1.65	.05–.10	(4)
	$A_2a_2\ Bb$ [3]	10.81	10.07	1.96	.20–.30	(5)
	A_1a_3 [2]	16.17	5.77	1.03	.10–.20	.10–.20

[1] Obtained ratio.
[2] Calculated on the basis of chromosome assortment.
[3] Calculated on the basis of random chromatid assortment.
[4] Total χ^2 cannot be calculated since both 35 : 1 and 15 : 1 ratios were expected and these cannot always be distinguished with the numbers of plants used.
[5] Total χ^2 cannot be calculated since 86.1 : 1, 20.8 : 1, and 12.9 : 1 ratios were expected and these cannot always be distinguished with the numbers of plants used.

The results in I_2 of Ti 6 (15) were similar to those for Ti 2 (11) (table 6). In this case, 22 I_2 lines averaging 125.6 seedlings per line were classified. The observed ratios did not fit satisfactorily either the hypothesis of dihybrid or trihybrid inheritance. In contrast to Ti 2 (11), the fit was satisfactory to the ratios expected from the genotype A_2a_2bb with chromatid assortment, but total χ^2 gave P of less than 0.01 on the assumption of chromosomal assortment. The fit was equally good for the hypothesis of duplex factors with hexasomic segregation (A_2a_4).

In the I_1 generation of Ti 15 (5) and Ti 11 (8), 632 and 557 seedlings, respectively, were classified and all were normal. Failure to obtain recessive seedlings in such large populations would occur very rarely if duplicate or triplicate factors with disomic inheritance were involved unless one or more of the genes were homozygous. Segregation in some of the I_2 lines in each family furnished evidence of heterozygosity for factors conditioning albino seedlings. Among the progeny of Ti 15 (5), 25 I_2 lines with an average of 79.4 seedlings per line were studied (table 6). The results substantiated the conclusion drawn from I_1 that neither dihybrid nor trihybrid ratios were involved. On the basis of the χ^2 test, it may be concluded that the fit was satisfactory of observed ratios to those expected from the genotype A_3a_1bb with chromosomal or chromatid segregation, the genotype A_2a_2Bb with chromosomal or chromatid segregation, or the genotype A_3a_3 with chromosomal assortment. Chromatid assortment from

the genotype A_2a_2Bb seems to be an unlikely explanation since in I_1 the deviation from expected on that basis gave P of less than 0.01.

The results obtained in I_2 of Ti 11 (8), in which 23 lines with 88.8 seedlings per line were studied, were similar to the results with Ti 15 (5). In one of the 9 segregating lines in this family, the observed ratio (127:14) deviated more than was expected by chance ($P<0.01$) from a 35:1, 20.8:1, or 24:1 ratio. The fit of this observed ratio to 15:1 was satisfactory ($P=0.05$ to 0.10). No ratio of this kind is expected in I_2 from the genotype A_3a_3 with chromosome assortment but ratios of 15:1 are expected in I_2 from the genotype A_2a_2Bb with chromosomal assortment, and ratios of 12.9:1 are expected in I_2 from A_2a_2Bb with chromatid segregation. Occasional I_2 lines segregating 15:1 also might be expected from a parent of the genotype A_2a_2bb since the occurrence of sexivalents indicated that all six homologous or homeologous chrosomes may pair. In such cases, a dominant allele could be transferred by crossing-over to one of the chromosomes normally carrying *bb*.

In the I_1 of Ti 16 (5) a ratio of 575 green : 4 albino was obtained. The deviations of this ratio from 143:1, 120:1, 86.1:1, or 63:1 were not statistically significant (table 5). In I_2, there were 5 lines not segregating to 18 segregating. None of the segregating lines showed a ratio approximating 3:1. The deviation from the expected on the basis of trihybrid inheritance was too great to be attributed to chance. Also, P was less than 0.01 for fit of the observed ratio to that calculated for the genotype A_2a_2Bb with chromatid segregation. Chi square for fit of observed to calculated for the genotype A_3a_3 with chromatid segregation gave P of 0.10 to 0.20.

DISCUSSION

The cytological results obtained in these investigations differ from those reported previously for hexaploid *Phleum pratense* in the high quadrivalent frequency and in the occurrence of sexivalents. In one plant, Myers (*12*) reported a low frequency of quadrivalents. However, the chiasma frequency of the plant was low, probably accounting for the low quadrivalent frequency. The disagreement with the results by Nordenskiöld (*16*) and Müntzing and Prakken (*10*) may have resulted from the different conditions under which the plants were grown or from differences in material. The plants studied by these authors probably were of European origin, whereas seven of the plants used in this investigation were from local collections in the northeastern part of the United States.

The plants used in these investigations had varying proportions of metaphase I sporocytes with univalents, anaphase I with laggards, and quartets with micronuclei. Such meiotic irregularities usually are expected in species with multivelent pairing. The differences among plants suggest the possibility of reducing the amount of irregularity in the species by selection. Similar differences among plants of *Dactylis glomerata* have been reported by Myers and Hill (*14, 15*).

The incidence of dicentric bridges and acentric fragments at anaphase I is in agreement with the reports of Müntzing (*9*), Myers and Hill (*14, 15*), Myers (*11, 12*), and Östergren (*18*), of the frequent

occurrence of chromosomal aberrations among plants of naturally cross-pollinated species.

Genetic results reported for *Phleum pratense* have been limited primarily to the first segregating generation. Geneticists have recognized generally that tests in the second segregating generation (I_2 or F_3) are much more sensitive than I_1 or F_2 tests Myers (*12*, *13*) has emphasized particularly the inadequacy of the I_1 test for distinguishing between disomic and polysomic inheritance. The types of chromosomal association that might be expected in a hexaploid have been outlined previously in this report. The high quadrivalent frequency observed in these plants provides cytological evidence against the hypothesis of allohexaploidy, and the genetic results were completely in agreement with the cytological in that regard so far as the genes studied in these experiments are concerned. The possibility cannot be ignored, of course, that the chromosomes may be differentiated to varying degrees so that one set will usually pair as three bivalents while the others associate at random in fours or sixes. Critical evidence on this point can be obtained only when marker genes are available for most or all of the chromosomes.

The quadrivalent frequency in the plants investigated was similar to that obtained in *Dactylis glomerata* (*14*, *15*), autotetraploid *Lolium perenne*,[5] unpublished, and several other autotetraploid grasses (*14*). This result together with the low incidence of sexivalents might be interpreted to indicate that there were four genomes of one kind and two of another instead of six homologous genomes. On this hypothesis, the occasional sexivalents could be explained on the basis of incomplete differentiation of chromosomes of the two genomes from those of the four. However, such cytological evidence is not critical. The two alternative hypotheses can be evaluated adequately only by genetic data. In the present investigations, the numbers of I_2 lines and the numbers of plants within lines were too small to afford a sensitive genetic test of the hypotheses. In addition to the use of larger populations, I_3 tests of selected I_2 lines would be valuable for such a test.

The results obtained in these investigations are of considerable importance to the plant breeder. In an allohexaploid, only monohybrid dihybrid, and trihybrid ratios are expected for simply inherited characters. Homozygosity of the dominant allele in one of the three genomes is effective in preventing further phenotypic segregation, while lines segregating in a monohybrid ratio will contain only plants that are homozygous and plants that produce progenies again segregating in a 3 : 1 ratio. In polyploids with six homologous genomes or with four genomes of one kind and two of another, the genetic results are much more complex. This fact is evident from the calculated ratios presented in tables 3 and 4. In a polyploid of either of these types, two, or in some cases three, generations of progeny tests would be required to prove that a plant was homozygous for any particular factor.

In naturally cross-pollinated species, such as *Phleum pratense*, inbreeding is the most effective method of obtaining uniformity for important characters. According to the calculations of Bartlett and Haldane (*2*), the rate of approach to homozygosity is expected to be

[5] See footnote 4.

very slow in autopolyploids. Therefore, more generations of inbreeding will be required in *P. pratense* than in an allohexaploid to attain the same degree of homozygosity. On the other hand, this characteristic of autopolyploids would be an advantage in the use of hybrids involving inbred lines for commercial production, since the reduction in vigor in advanced generations of the hybrids should be less rapid than with diploids or allopolyploids.

SUMMARY

In the plants of hexaploid *Phleum pratense L.* that were studied, the chromosomes at diakinesis were associated as bivalents, quadrivalents, and sexivalents. Among seven plants, the average number of quadrivalents per sporocyte ranged from 2.9 to 4.9 and the average number of sexivalents from 0 to 0.9. In any sporocyte the maximum number of quadrivalents observed was 7 and of sexivalents 3. Chiasma frequency was not correlated with multivalent frequency in these plants.

The plants had varying percentages of univalents at metaphase I, lagging and dividing chromosomes, dicentric bridges, and acentric fragments at anaphase I, and micronuclei in the quartets.

Genetic data from the I_1 and I_2 generations of five plants were in agreement with the cytological results in that dihybrid and trihybrid ratios were not obtained, indicating that the chromosomes did not pair regularly as particular bivalents. The genetic data were insufficient to afford critical evidence as to whether there are six homologous genomes or four genomes of one kind and two of another in *Phleum pratense.*

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EFFECT OF HEAT TREATMENT AND OIL EXTRACTION ON THE UTILIZATION AND DIGESTIBILITY OF SOYBEAN PROTEIN BY LAMBS[1]

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INTRODUCTION

Soybeans and soybean oil meal have become increasingly important as livestock feeds in this country. With the recent great increase in the acreage of soybeans because of the demand for soybean oil, the availability of soybean oil meal for livestock feeding will be even greater. The utilization of this soybean protein to the best advantage is of considerable importance to the livestock industry.

Various experiments with nonruminants, such as pigs, chickens, and rats, have shown that both raw soybeans and soybean oil meals that have not been well cooked furnished protein of poor quality or low value when fed as the principal source of protein (*2, 5, 9, 4, 11, 12, 13, 15, 16, 20, 21.*)[3] On the other hand, these experiments have shown that well-cooked soybean oil meals furnish good-quality protein, and that heat treatment of raw soybeans, or further heat treatment of insufficiently cooked soybean oil meals, results in marked improvement in the efficiency of the protein.

The improvement of the soybean protein by heat treatment was shown by some increase in digestibility and considerable increase in efficiency. In general, for rats the heated soybean protein had a digestibility from 3 to 7 percent greater than that of raw soybean protein (*2, 4, 16, 20*). The biological values for rats reported by Hayward et al. (*2*) averaged 41 for raw soybean protein and 51 for heated soybean protein when each was fed at an 18-percent level of protein. Mitchell and Beadles (*4*) give biological values of 49 for raw soybean protein and 67 for heated soybean protein when fed to rats at a 10-percent level of protein, as reported by the Illinois station (*4, pp. 93–95*).

Certain investigators have found that cystine supplements raw soybean protein, thereby greatly improving its value in rations for rats (*9, 4, 16*). Mitchell and Beadles (*4*) found that cystine also supplements cooked soybean protein. However, when the cystine deficiencies of both the raw and heated soybean proteins were corrected, heated soybeans were still superior for rats. These investigators concluded that heating makes not only cystine but also probably certain other amino acids more available to rats.

Few nitrogen-balance experiments or digestion trials to measure the effect of heat treatment on the nutritive value and digestibility of soybean protein have been conducted with ruminants. Scharrer

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[3] Italic numbers in parentheses refer to Literature Cited, p. 47.

and Nebelsiek (*14*) found that for sheep the coefficients of digestibility of the various nutrients of steamed soybeans were higher than those for raw soybeans. Hamilton and Kammlade (*3, pp. 73–76*), at the Illinois station, found no difference in the digestibility of the protein of soybeans and soybean oil meal when fed to lambs.

As summarized by Morrison (*10*), feeding experiments have shown that raw soybeans and well-cooked soybean oil meal are of about equal value as protein supplements to rations for dairy cattle and sheep. For all beef cattle, except fattening calves, soybeans have been found equal in feeding value to soybean oil meal. There has been little or no difference for ruminants in the value of the protein furnished by soybean oil meals prepared by different methods. In Indiana experiments King [4] found no advantage in cooking soybeans for fattening cattle.

OBJECTS OF EXPERIMENTS

The objects of the experiments reported in this paper were (1) to determine the effect of heat treatment on the digestibility and nutritive value of soybean protein for lambs, and (2) to determine whether or not the addition of soybean oil to a soybean oil meal ration, in an amount equal to that contained in a soybean ration, would affect the ability of lambs to digest or utilize protein.

EXPERIMENTAL PROCEDURE

Nitrogen-balance experiments were conducted on wether lambs of good mutton type. The urine and feces were collected daily by means of metabolism cages during 10-day collection periods. All preliminary or intervening periods were at least 10 days in length. The general procedure and methods of analysis were practically the same as those followed in the earlier work reported by this station (*18, 19*).

The data on digestibility are expressed as apparent digestibility and as estimated true digestibility. The efficiency of nitrogen utilization is expressed as percentage storage of total nitrogen, percentage storage of digested nitrogen, and biological value. No direct determinations of endogenous nitrogen losses in the urine and metabolic nitrogen losses in the feces were made on these lambs. Experimental work conducted at this station (*8*) has indicated that low-nitrogen periods of feeding, necessary for making these determinations, cause unduly high nitrogen utilization by lambs when subsequently fed the test rations. Therefore, as in earlier work (*6, 7*), average values for metabolic nitrogen losses and endogenous nitrogen losses, as determined at this station on comparable lambs, were used. The average values were 0.037 gm. of endogenous nitrogen daily per kilogram of body weight and 0.55 gm. of metabolic nitrogen per 100 gm. of dry-matter intake. The soundness of these average values has been strengthened by a recent paper of Harris and Mitchell (*1*). Using a similar low-nitrogen basal ration, they report average values for lambs of 0.033 gm. of endogenous nitrogen daily per kilogram of body weight and 0.555 gm. of metabolic nitrogen per 100 gm. of dry-matter intake.

[4] KING, F. G. Ind. Agr. Expt. Sta. Mimeo. Rpts. (Unpublished data.)

EXPERIMENT 1

The four soybean feeds used in experiment 1 were raw soybeans, soybean flakes, solvent-process soybean oil meal, and heat-treated solvent-process soybean oil meal. These feeds were furnished by a Corn Belt soybean oil mill, and were from the same batch of soybeans.

The raw soybeans were of good quality, and presumably were typical of soybeans marketed in the Corn Belt. By analysis, they contained 35.1 percent of crude protein and 18.9 percent of ether extract.

The soybean flakes were a product resulting from the initial processing of soybeans in the solvent-extraction method. The beans were first dried at a maximum temperature of 180° F. for approximately 20 minutes. Then the dried beans were cracked, heated to a maximum temperature of 165° F. for 15 minutes, and flaked. Therefore, the soybean flakes as fed in this experiment were unextracted and had received only sufficient heat treatment for efficient flaking. They were light in color and had the same crude-protein and ether-extract content as the raw soybeans. This product should not be confused with extracted soybean flakes, which receive more heat treatment.

The solvent-extracted soybean oil meal was produced by the commercial process of extracting soybeans with hexane. It received only sufficient heat for drying the beans, flaking therm, and removing the hexane. Frequently, such meal has been referred to as white soybean oil meal or raw soybean oil meal. In the extraction process, the soybean flakes described above were held at a temperature of approximately 125° F. for 30 minutes, and the extracted residue was then heated to a maximum of 200° for 20 minutes to drive off the solvent. The resulting meal contained 44.6 percent of crude protein and 0.9 percent of ether extract.

The heat-treated solvent-process soybean oil meal was produced by further heating or "toasting" of the extracted meal for 70 minutes at a maximum temperature of approximately 250° F. This meal was light brownish or tan in color. The crude-protein content was 42.1 percent and the ether-extract content was 0.9 percent.

The low-nitrogen basal ration used in this experiment was the low-nitrogen ration for lambs developed at this station for use in determining biological values of proteins.[1] It was composed of purified cellulose, starch, brown sugar, corn oil, minerals, and wheat straw. The total protein content averaged about 1.0 percent. Numerous nitrogen-balance trials have shown this basal ration to be entirely inadequate to support either nitrogen equilibrium or maintenance of body weight.

The composition of each ration as fed is given in table 1.

Each of the soybean products was included in sufficient amounts to furnish 10 percent total protein in its respective ration. The lambs were fed twice daily and each ration was fed as a complete mixture. The order in which each lamb was fed each of the four rations was varied in such a manner as to make an orderly rotation.

The data of experiment 1 are presented in table 2 and the values for the digestibility and the utilization of the nitrogen are summarized in table 3.

TABLE 1.—*Composition of the rations fed in experiment 1*

Ingredients	Ration containing—			
	Raw ground soybeans	Soybean flakes	Solvent-process soybean oil meal without special heat treatment	Solvent-process soybean oil meal with special heat treatment
	Percent	*Percent*	*Percent*	*Percent*
Soybean product	28.50	28.50	22.43	23.75
Cellulose	9.00	9.00	9.00	9.00
Wheat straw	25.00	25.00	25.00	25.00
Starch	17.25	17.25	19.28	18.62
Sugar	17.25	17.25	19.29	18.63
Corn oil			2.00	2.00
Mineral [1]	3.00	3.00	3.00	3.00
Average total protein	10.41	10.48	10.96	11.09
Average fat	5.77	5.77	2.58	2.59

[1] The mineral mixture was composed of 40 percent ground limestone, 40 percent steamed bonemeal, and 20 percent salt.

TABLE 2.—*Nitrogen-balance data showing digestibility and utilization of nitrogen in experiment 1.*[1]

GROUND SOYBEAN RATION

Number of lamb	Weight of lamb			Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Total nitrogen stored	Apparent digestibility	True digestibility (estimated)	Digested nitrogen stored	Biological value
	Initial	Final	Average										
	Kg.	*Kg.*	*Kg.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	
1	27.6	27.3	27.4	8,000	7,368.8	134.19	56.55	57.06	15.3	57.5	87.8	26.7	61
2	30.9	31.1	31.0	8,000	7,356.4	133.96	58.61	52.10	17.4	61.1	91.4	28.4	62
3	27.8	27.3	27.6	6,000	5,486.7	99.91	36.53	41.41	22.0	58.6	88.9	37.6	70
4	22.5	23.1	22.8	7,000	6,405.0	116.64	53.26	57.03	5.4	51.1	81.4	10.6	53
5	28.5	27.8	28.2	8,000	7,330.0	133.48	72.64	53.82	5.3	59.7	90.0	8.8	48
6	27.8	28.0	27.9	8,000	7,293.6	132.82	70.85	38.26	17.8	71.2	100.0	25.1	54
7	32.3	32.3	32.3	8,000	7,293.6	132.82	62.50	48.91	16.1	63.2	93.5	25.5	59
8	31.9	32.6	32.2	8,000	7,318.8	133.28	65.62	40.14	20.6	69.9	100.0	29.5	60
SOYBEAN FLAKE RATION													
1	29.2	29.9	29.6	8,000	7,274.0	132.97	62.09	46.89	18.0	64.7	94.9	27.9	59
2	34.9	35.0	35.0	8,000	7,300.8	133.46	61.95	38.46	24.8	71.2	100.0	34.8	63
3	27.8	28.4	28.1	8,000	7,377.2	134.86	55.66	56.22	17.0	58.3	88.5	29.2	62
4	25.3	25.8	25.6	8,000	7,327.2	133.94	54.21	56.32	17.5	58.0	88.1	30.2	62
5	24.1	24.4	24.2	6,000	5,501.1	100.56	58.44	34.52	7.6	65.7	95.9	11.5	49
6	24.1	25.3	24.7	7,000	6,427.4	117.49	61.38	43.53	10.7	63.0	93.2	17.0	52
7	30.4	31.0	30.7	8,000	7,319.2	133.80	60.20	47.81	19.3	64.3	94.5	30.0	61
8	30.7	30.7	30.7	8,000	7,274.0	132.97	60.93	51.47	15.5	61.3	91.5	25.2	59
SOYBEAN OIL MEAL RATION													
1	28.1	29.0	28.6	8,000	7,340.4	140.35	70.13	38.36	22.7	72.7	100.0	31.2	58
2	32.4	33.2	32.8	8,000	7,393.2	139.45	71.01	50.22	13.1	64.0	92.9	20.4	55
3	31.8	32.3	32.0	8,000	7,293.2	139.45	63.61	39.37	26.2	71.8	100.0	36.4	63
4	28.5	29.9	29.2	8,000	7,376.0	141.03	53.91	41.72	32.2	70.4	99.3	45.7	69
5	26.2	26.1	26.2	8,000	7,337.2	140.29	60.05	49.37	22.0	64.8	93.7	34.0	62
6	26.2	27.4	26.8	8,000	7,306.4	139.70	69.83	49.96	14.2	64.2	93.1	22.2	54
7	24.6	25.2	24.9	6,000	5,520.9	105.56	53.33	35.94	15.4	66.0	94.8	23.4	56
8 [2]	27.3	25.0	26.2	5,103	4,699.6	89.86	48.80	40.26	.9	55.6	84.1	1.2	48
HEAT-TREATED SOYBEAN OIL MEAL RATION													
1	25.5	25.8	25.6	6,000	5,524.8	106.90	58.10	35.61	12.3	66.7	95.2	18.5	52
2	28.4	28.6	28.5	7,000	6,455.0	124.90	64.06	35.85	20.0	71.3	99.8	28.1	57
3	30.4	30.9	30.6	8,000	7,278.4	140.84	73.93	44.84	14.9	68.2	96.7	23.0	54
4	26.4	27.5	27.0	8,000	7,248.8	140.26	59.44	45.54	25.2	67.5	96.1	37.2	63
5	28.5	29.7	29.1	8,000	7,248.8	140.26	53.22	41.13	32.7	70.7	99.2	46.3	69
6	29.1	30.0	29.6	8,000	7,388.4	142.97	58.30	47.63	25.9	66.7	95.2	38.9	65
7	27.2	28.4	27.8	8,000	7,367.2	142.56	64.43	43.97	24.0	69.2	97.7	34.6	61
8	28.7	29.2	29.0	8,000	7,440.0	143.96	54.48	50.73	26.9	64.8	93.3	41.6	67

[1] Totals represent 10-day experimental periods.
[2] Data not included in summary of results because of low feed intake and loss of weight.

TABLE 3.—*Summary of digestibility and utilization of nitrogen in experiment 1*

Item	Data for lamb No. —								Average [1]
	1	2	3	4	5	6	7	8	
Ground soybean ration:									
Apparent digestibility.....percent	57.5	61.1	58.6	51.1	59.7	71.2	63.2	69.9	61.5±1.57
True digestibility.....do	87.8	91.4	88.9	81.4	90.0	100.0	93.5	100.0	91.6±1.49
Total nitrogen stored.....do	15.3	17.4	22.0	5.4	5.3	17.8	16.1	20.6	15.0±1.51
Digestible nitrogen stored.....do	26.7	28.4	37.6	10.6	8.8	25.1	25.5	29.5	24.0±2.31
Biological value	61	62	70	53	48	54	59	60	58 ±1.60
Soybean flake ration:									
Apparent digestibility.....percent	64.7	71.2	58.3	58.0	65.7	63.0	64.3	61.3	63.3±1.03
True digestibility.....do	94.9	100.0	88.5	88.1	95.9	93.2	94.5	91.5	93.3± .94
Total nitrogen stored.....do	18.0	24.8	17.0	17.5	7.6	10.7	19.3	15.5	16.3±1.25
Digestible nitrogen stored.....do	27.9	34.8	29.2	30.2	11.5	17.0	30.0	25.2	25.7±1.84
Biological value	59	63	62	62	49	52	61	59	58 ±1.23
Soybean oil meal ration:									
Apparent digestibility.....percent	72.7	64.0	71.8	70.4	64.8	64.2	66.0	.....	67.7± .96
True digestibility.....do	100.0	92.9	100.0	99.3	93.7	93.1	94.8	.....	96.3± .86
Total nitrogen stored.....do	22.7	13.1	26.2	32.2	22.0	14.2	15.4	.....	20.8±1.79
Digestible nitrogen stored.....do	31.2	20.4	36.4	45.7	34.0	22.2	23.4	.....	30.5±2.33
Biological value	58	55	63	69	62	54	56	.....	60 ±1.38
Heat-treated soybean oil meal ration:									
Apparent digestibility.....percent	66.7	71.3	68.2	67.5	70.7	66.7	69.2	64.8	68.1± .52
True digestibility.....do	95.2	99.8	96.7	96.1	99.2	95.2	97.7	93.3	96.6± .52
Total nitrogen stored.....do	12.3	20.0	14.9	25.2	32.7	25.9	24.0	26.9	22.7±1.59
Digestible nitrogen stored.....do	18.5	28.1	23.0	37.2	46.3	38.9	34.6	41.6	33.5±2.28
Biological value	52	57	54	63	69	65	61	67	61 ±1.47

[1] The error calculated is the probable error of the mean.

The apparent digestibility of the nitrogen averaged 61.5 percent for the raw soybean ration, 63.3 percent for the soybean flake ration, 67.7 percent for the solvent-process soybean oil meal ration, and 68.1 percent for the toasted soybean oil meal ration. These data show an appreciably lower digestibility for the raw soybean protein and the soybean flake protein than for the protein in either of the soybean oil meal rations. The average values for the estimated true digestibility of the nitrogen were also higher for the two rations containing the meals than for the rations containing either the raw soybeans or the soybean flakes. Further heat treatment of the solvent-process soybean oil meal resulted in little or no improvement in digestibility.

The lambs stored nearly the same percentage of the total nitrogen intake when fed either the heat-treated soybean oil meal ration or the ration with the soybean oil meal without special heat treatment. The average retention was 22.7 percent and 20.8 percent, respectively. Also, the lambs stored nearly the same percentage of the total nitrogen intake when fed either the ground soybean ration or the soybean flake ration, but the storage on each was lower than when the soybean oil meals were fed.

The percentage retention of the digested nitrogen was greatest by the lambs while they were fed the heat-treated soybean oil meal ration, or 33.5 percent. The lambs stored 30.5 percent, or only slightly less, when fed the ration with soybean oil meal without special heat treatment. The retention of digested nitrogen was appreciably less by the lambs when fed the ground soybeans and the soybean flakes, being 24.0 percent and 25.7 percent, respectively.

There was little difference in the average biological values of the four rations. The values, however, were in the same order as the values obtained on the percentage retention of digested nitrogen which did not consider the endogenous and metabolic nitrogen losses.

EXPERIMENT 2

Nitrogen-balance experiments were conducted on another group of eight lambs in a further study of the effect of heat treatment and oil extraction on the digestibility and utilization of soybean protein. Ground raw soybeans, solvent-process soybean oil meal, and solvent-process soybean oil meal with special heat treatment, similar in all respects to the products used in experiment 1, were employed. The soybean flake ration was not tested in this series, as it was believed that the first experiment had amply demonstrated that the nitrogen of soybean flakes had about the same digestibility and utilization as the nitrogen in raw soybeans. Instead, a ration was fed in which soybean oil was added with heat-treated soybean oil meal in the amount needed to bring the total fat content up to that of the ground soybean ration. Refined soybean oil was used for this purpose as lambs failed to consume well a ration containing the needed amounts of crude soybean oil. The low-nitrogen basal ration in this experiment was the same as that used in the first experiment, except that soybean oil replaced corn oil in the basal ration.

The percentage composition of the rations fed is shown in table 4.

TABLE 4.—*Composition of the rations fed in experiment 2*

Ingredients	Ration containing—			
	Raw ground soybeans	Solvent-process soybean oil meal without special heat-treatment	Solvent-process soybean oil meal with special heat-treatment	Solvent-process soybean oil meal with special heat-treatment plus soybean oil
	Percent	*Percent*	*Percent*	*Percent*
Soybeans or soybean oil meal	28.22	23.60	21.94	21.94
Cellulose	9.0	9.0	9.0	9.0
Wheat straw	25.0	25.0	25.0	25.0
Starch	17.39	19.20	19.53	17.84
Sugar	17.39	19.20	19.53	17.84
Soybean oil		2.0	2.0	5.38
Mineral [1]	3.0	3.0	3.0	3.0
Average total protein	10.50	11.44	11.25	11.31
Average fat	5.94	2.50	2.56	5.94

[1] The mineral mixture was composed of 40 percent ground limestone, 40 percent steamed bonemeal, and 20 percent salt.

The detailed results of experiment 2 are given in table 5 and the data on digestibility and utilization of the nitrogen are summarized in table 6.

The results of this experiment check closely with the results obtained in the first experiment. In general, values for both the digestibility and the utilization of the nitrogen tended to be higher than those in experiment 1.

The apparent digestibility and the estimated true digestibility of the nitrogen in the raw soybean ration averaged lower than for the other rations. The apparent digestibility of the nitrogen averaged 64.3 percent as compared with 70.4 percent for the solvent-process soybean oil meal ration and 73.1 for the treated soybean oil meal ration. There was no significant difference in the digestibility of the nitrogen between the ration containing the solvent-process meal without special heat treatment and the ration with the toasted meal.

The addition of soybean oil to the ration containing heat-treated soybean oil meal, in the amount needed to give the same fat content as in the raw soybean ration, had a slightly depressing effect on the digestibility of the nitrogen. This difference was not sufficient to be statistically significant.

TABLE 5.—*Nitrogen-balance data showing digestibility and utilization of nitrogen in experiment 2* [1]

Number of lamb	Weight of lamb			Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Total nitrogen stored	Apparent digestibility	True digestibility (estimated)	Digestible nitrogen stored	Biological value
	Initial	Final	Average										
	Kg.	*Kg.*	*Kg.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	
GROUND SOYBEAN RATION													
1	32.8	32.7	32.8	7,867	7,237.6	131.65	58.58	46.60	20.1	64.6	95.0	31.1	63
2	32.8	33.2	33.0	8,000	7,375.2	134.15	63.89	55.81	10.8	58.4	88.7	18.4	56
3	30.4	31.6	31.0	7,000	6,469.4	117.68	50.33	30.08	31.7	74.4	100.0	42.6	67
4	27.8	28.0	27.9	7,000	6,469.4	117.68	48.92	45.82	19.5	61.1	91.4	31.9	64
5	30.2	30.0	30.1	8,000	7,376.8	134.18	57.86	46.88	21.9	65.1	95.4	33.7	64
6	27.5	27.4	27.4	7,000	6,444.2	117.22	52.53	42.16	19.2	64.0	94.4	30.0	62
7	25.2	25.9	25.6	7,000	6,429.5	116.95	45.47	45.12	22.5	61.4	91.8	36.7	66
8	25.9	25.7	25.8	5,650	5,189.5	94.40	37.14	32.40	26.3	65.7	96.0	40.1	70
SOYBEAN OIL MEAL RATION													
1	30.6	31.2	30.9	7,095	6,523.1	130.27	63.04	34.42	25.2	73.6	100.0	34.2	60
2	31.2	32.0	31.6	8,000	7,355.2	146.88	57.74	45.14	30.0	69.3	96.9	43.2	68
3	34.4	36.0	35.2	8,000	7,382.4	147.43	64.92	34.13	32.8	76.8	100.0	42.7	65
4	30.7	30.7	30.7	8,000	7,376.0	147.30	67.80	47.08	22.0	68.0	95.7	32.4	60
5	26.5	27.7	27.1	7,000	6,435.8	128.52	47.66	47.02	26.3	63.4	91.1	41.5	68
6	23.9	24.4	24.2	5,650	5,209.9	104.04	48.45	28.09	26.4	73.0	100.0	36.2	62
7	27.1	27.2	27.2	7,000	6,454.7	128.90	58.41	40.49	23.3	68.6	96.2	33.9	61
8 [2]	26.0	25.8	25.9	5,249	4,832.2	96.50	44.22	11.10	42.7	88.5	98.4	46.6	67
HEAT TREATED SOYBEAN OIL MEAL RATION—LOW FAT													
1	27.3	27.7	27.5	7,000	6,396.6	125.76	60.23	35.20	24.1	72.0	100.0	33.5	60
2	26.7	27.7	27.2	7,000	6,442.8	126.66	64.57	32.16	23.6	74.6	100.0	31.7	57
3	32.4	33.8	33.1	8,000	7,370.4	144.90	54.68	35.17	38.0	75.7	100.0	50.2	71
4	29.6	30.5	30.0	8,000	7,268.8	142.90	53.86	38.56	35.3	73.0	100.0	48.4	70
5	29.1	29.0	29.0	7,000	6,476.4	127.33	53.21	36.85	29.3	71.1	99.1	41.2	66
6	26.1	26.8	26.4	7,000	6,476.4	127.33	48.80	36.07	33.4	71.7	99.8	46.5	69
7	31.2	31.4	31.3	7,640	6,941.7	136.47	62.43	41.56	23.8	69.5	97.6	34.2	62
8	30.3	31.1	30.7	8,000	7,337.6	144.26	65.99	32.87	31.5	77.2	100.0	40.8	62
HEAT TREATED SOYBEAN OIL MEAL RATION—HIGH FAT													
1	28.6	29.2	28.9	7,000	6,493.2	127.14	54.88	39.50	25.8	68.9	97.1	37.4	64
2	29.3	30.0	29.6	7,000	6,493.2	127.14	44.59	38.72	34.5	69.5	97.7	49.6	73
3	29.1	29.6	29.4	7,000	6,433.0	125.96	52.80	33.42	31.6	73.5	100.0	42.9	67
4	26.0	27.7	26.8	7,000	6,482.0	126.92	41.35	36.43	38.7	71.3	99.5	54.3	75
5	31.0	31.8	31.4	8,000	7,369.6	144.30	70.40	36.17	26.2	74.9	100.0	34.9	59
6	28.9	29.7	29.3	6,519	6,014.4	117.76	49.44	33.09	29.9	71.9	100.0	41.6	67
7	30.0	30.3	30.2	8,000	7,366.4	144.23	57.86	49.05	25.9	66.0	94.2	39.2	66
8	26.9	27.8	27.4	7,000	6,448.4	126.26	41.55	34.24	40.0	72.9	100.0	54.8	75

[1] Total represent 10-day experimental periods.
[2] Data not included in summary of results.

The percentage of the total nitrogen stored by the lambs also averaged lower for the soybean ration. The average for this ration was 21.5 percent as compared with 26.6 percent for the soybean oil meal ration without special heat treatment, 29.9 percent for the

toasted meal ration, and 31.6 percent for the toasted meal high-fat ration. The percentage of digested nitrogen stored, uncorrected for endogenous and metabolic nitrogen losses, averaged 33.1, 37.7, 40.8, and 44.3, respectively.

TABLE 6.—*Summary of digestibility and utilization of nitrogen in experiment 2*

Item	Data for lamb No.—								Average [1]
	1	2	3	4	5	6	7	8	
Ground soybean ration:									
Apparent digestibility.....percent..	64.6	58.4	74.4	61.1	65.1	64.0	61.4	65.7	64.3±1.13
True digestibility.....do....	95.0	88.7	100.0	91.4	95.4	94.4	91.8	96.0	94.1± .82
Total nitrogen stored.....do....	20.1	10.8	31.7	19.5	21.9	19.2	22.5	26.3	21.5±1.44
Digestible nitrogen stored.....do....	31.1	18.4	42.6	31.9	33.7	30.0	36.7	40.1	33.1±1.77
Biological value.....	63	56	67	64	64	62	66	70	64 ± .98
Soybean oil meal ration:									
Apparent digestibility.....percent..	73.6	69.3	76.8	68.0	63.4	73.0	68.6	.....	70.4±1.13
True digestibility.....do....	100.0	96.9	100.0	95.7	91.1	100.0	96.2	.....	97.1± .83
Total nitrogen stored.....do....	25.2	30.0	32.8	22.0	26.3	26.4	23.3	.....	26.6± .96
Digestible nitrogen stored.....do....	34.2	43.2	42.7	32.4	41.5	36.2	33.9	.....	37.7±1.17
Biological value.....	60	68	65	60	68	62	61	.....	63 ± .91
Heat-treated soybean oil meal ration—low fat:									
Apparent digestibility.....percent..	72.0	74.6	75.7	73.0	71.1	71.7	69.5	77.2	73.1± .61
True digestibility.....do....	100.0	100.0	100.0	100.0	99.1	99.8	97.6	100.0	99.6± .20
Total nitrogen stored.....do....	24.1	23.6	38.0	35.3	29.3	33.4	23.8	31.5	29.9±1.34
Digestible nitrogen.....do....	33.5	31.7	50.2	48.4	41.2	46.5	34.2	40.8	40.8±1.71
Biological value.....	60	57	71	70	66	69	62	62	65 ±1.23
Heat-treated soybean oil meal ration—high fat:									
Apparent digestibility.....percent..	68.9	69.5	73.5	71.3	74.9	71.9	66.0	72.9	71.1± .68
True digestibility.....do....	97.1	97.7	100.0	99.5	100.0	100.0	94.2	100.0	98.6± .50
Total nitrogen stored.....do....	25.8	34.5	31.6	38.7	26.2	29.9	25.9	40.0	31.6±1.36
Digestible nitrogen stored.....do....	37.4	49.6	42.9	54.3	34.9	41.6	39.2	54.8	44.3±1.82
Biological value.....	64	73	67	75	59	67	66	75	68 ±1.36

[1] The error calculated is the probable error of the mean.

There was somewhat less difference in the biological values than in the values for the uncorrected storage of digested nitrogen. The average values were 64, 63, 65, and 68, for the ground soybeans, solvent meal, heat-treated meal, and the heat-treated meal plus soybean oil, respectively.

A further comparison of the soybean ration and the solvent-process soybean oil meal ration was made as a part of a third experiment, as yet unpublished, which was initially undertaken to study another problem. This experiment had to be terminated because the corn silage fed in the ration became unfit for use; the opportunity was then taken to obtain additional information, even though limited, on the soybean and the soybean oil meal rations. Analysis of the data obtained reveals that the lambs responded somewhat more poorly to each ration than the lambs in the two experiments reported in detail in this paper. This was probably due to their being off feed for some time because of the poor quality of the corn silage previously fed. The results obtained for the two rations differ somewhat from those obtained in the other experiments as there was no difference in the digestibility of the nitrogen, and the soybean ration had an unexpectedly low average biological value, being 52 as compared with 62 for the soybean oil meal ration. The fact that unthrifty lambs responded differently from thrifty lambs when fed the soybean ration may possess some significance. However, because of the nature of the experiment, these data were not considered as reliable as the

other data herein reported and therefore were not considered in the summary of results.

DISCUSSION

All of the rations fed in these experiments seemed to be palatable to the lambs. Little difficulty was experienced in making abrupt changes from one ration to another in either of the experiments.

The data of experiment 1 and experiment 2 on the digestibility and the utilization of the nitrogen in the rations with ground soybeans, solvent-process soybean oil meal, and solvent-process meal with special heat treatment, have been combined and treated statistically for significance by the analysis of variance method (*17*). Treatment of these data in this fashion indicates certain highly significant differences.

The percentage of apparent digestibility of nitrogen for the two experiments averaged 62.9, 69.0, and 70.6 for the ground soybeans, solvent-process soybean oil meal, and the solvent-process soybean oil meal with special heat treatment, respectively. Statistical treatment of these data indicates that the difference in the digestibility of the nitrogen between the ration with the solvent-process meal and the ration with the toasted solvent-process meal was so small that it might well have been due to chance variation. However, each of the soybean oil meal rations was sufficiently higher in the digestibility of the nitrogen as compared with the soybean ration to give highly significant odds against such a difference being due to chance.

It is of interest to note that the lambs digested the nitrogen of the two solvent-process soybean oil meal rations with about the same efficiency as reported earlier by this station for an expeller-process soybean oil meal ration (*8*). In the earlier experiment, which involved four lambs, an average apparent digestibility of 71 percent was found for the nitrogen in a ration similar to those fed in this experiment, except that it contained expeller-process soybean oil meal. These data indicate that solvent-process soybean oil meal and expeller-process soybean oil meal have approximately the same apparent digestibility of nitrogen for lambs.

The percentage of total nitrogen stored for the two experiments averaged 18.2, 23.7, and 26.3, for the soybean ration, solvent meal ration, and the heat-treated meal ration, respectively. Again, statistical treatment indicates no significant difference between the two soybean oil meal rations, but each ration permitted a significantly greater storage of the total nitrogen than the soybean ration.

The differences in the percentage of digested nitrogen stored were not so consistent between rations as were the differences in the percentage storage of total nitrogen. The percentage storage of digested nitrogen gave average values of 28.5, 34.1, and 37.2 for the soybean ration, the solvent soybean oil meal ration, and the heat-treated soybean oil meal ration, respectively. Analysis of variance shows that the difference between the value for the soybean ration and either of the soybean oil meal rations was significant at the 5-percent level but not at the 1-percent level.

The biological values averaged 61, 62, and 63, respectively, for the soybean ration, the solvent meal ration, and the heat-treated meal

ration. Statistical treatment showed no significant differences in the biological values.

These results indicate that for growing lambs, the most significant difference between raw soybean protein and solvent-process soybean oil meal protein is in digestibility. The raw soybean protein was about 91 percent as digestible as the protein in the meals. Further heat treatment of the solvent-process meal resulted in little or no improvement in digestibility of the nitrogen by the lambs.

These data indicate a possible difference in the ability of the lambs to utilize the digested nitrogen of the raw soybean as compared with that of the solvent process meals. However, there was no significant difference between the three rations in the biological values. Therefore, while the lambs utilized the digested raw soybean nitrogen somewhat less efficiently than the digested nitrogen of the solvent-process meal, further work would be desirable before definite conclusions were drawn that this was a significant difference.

The idigestibility and utilization of the nitrogen in soybean flakes were s milar to the values for the raw soybeans, though slightly higher. The small differences were not statistically significant. Apparently, the amount of heat involved in flaking was insufficient to affect the protein to any appreciable degree.

The ration with toasted soybean oil meal and sufficient soybean oil to give the same fat content as the soybean ration had a slightly lower digestibility of nitrogen than the toasted soybean oil meal ration without added fat. The average values for the eight lambs were 71.1 percent and 73.1 percent, respectively. While this difference is small, in six out of the eight comparisons the high-fat ration was lower in digestibility of nitrogen than the low-fat ration. Statistical treatment by the analysis of variance method shows this difference to be somewhat too small for significance.

Since the experiments reported in this paper showed some differences in the digestibility of protein between rations, the question arose as to whether or not there might be differences in the digestibility of other nutrients. A complete digestibility study was not made of the various nutrients as these experiments were conducted solely to study the protein. However, since the air-dry weight of feces produced by the lambs on the different rations varied considerably, differences in the digestibility of the dry matter undoubtedly had occurred.

The dry-matter content of the feces was not determined as a part of these experiments and not all feces samples were still available when the data were summarized. In another experiment, the moisture content of 28 feces samples prepared for analysis in the same manner had an average dry-matter content of 92.5 percent. Using this average value, the digestibility of the dry matter was calculated for each ration in the two experiments reported in this paper.

In experiment 1, the average digestibility of the dry matter was 64 percent for the soybean ration, 66 percent for the soybean flake ration, 70 percent for the solvent-process soybean oil meal ration, and 70 percent for the toasted soybean oil meal ration. In experiment 2, the average values were 65 percent for the soybean ration, 69 percent for the solvent-process soybean oil meal ration, 70 percent for the toasted soybean oil meal ration, and 66 percent for the toasted soybean oil meal ration with added fat.

These data indicate that the digestibility of the dry matter was lower by the lambs when fed the soybean and the soybean flake rations than when fed either of the soybean oil meal rations. Further heat treatment of the solvent-process soybean oil meal did not affect the digestibility of the dry matter. The high-fat toasted soybean oil meal ration was significantly less digestible than the toasted soybean oil meal ration without the additional fat and of nearly the same digestibility as the soybean and soybean flake rations. These results indicate that the fat content of the soybeans and soybean flakes was largely responsible for the lower digestibility of the dry matter of these rations as compared with the soybean oil meal rations.

The effect of fat content on the digestibility of the dry matter was apparently more marked than on the digestibility of the protein alone, since the digestibility of the protein in the toasted meal, high-fat ration was slightly, but not significantly, lower than that of the toasted meal ration without the additional fat. Therefore, the higher digestibility of the soybean oil meal protein as compared with raw soybean protein was probably due both to the heat treatment and to the removal of the oil, but more largely to the heat treatment.

The presence or absence of soybean oil seemed to have little effect on the use lambs made of the digested soybean protein. In experiment 2, the soybean oil meal ration with added fat furnished nitrogen at least equal in efficiency to that of a similar soybean oil meal ration without the additional fat.

These data with lambs are similar to most of the data obtained with rats in showing that heat treatment improves the digestibility of soybean protein. However, these data differ in regard to the effect of heat treatment on the efficiency of the protein. Lambs do not utilize the heated protein with much, if any, more efficiency than raw soybean protein, whereas rats utilize the heated protein with considerably more efficiency.

Nitrogen-balance data at this station and elsewhere have shown that ruminants are less specific in their requirements for the commonly accepted essential amino acids than nonruminants. This has led to the belief that bacteria in the rumen synthesize nutritively complete protein from less complete feed protein, or from simple forms of nitrogen, and eventually this bacterial protein is available to the animal.

Before drawing practical applications from these experiments, it should be borne in mind that the soybean products furnished nearly all the protein in the experimental rations. Therefore, any differences between these sources of protein were magnified as compared with differences that might be expected in practical feed lot rations. For example, in lamb-feeding experiments now in progress at this station to compare ground soybeans and soybean oil meal, as well as other protein-rich feeds, as protein supplements to a basal ration of corn, corn silage, and minerals, the supplements furnished about 26 percent of the total protein in the rations. In similar feeding experiments with yearling steers, the supplements furnished approximately 30 percent of the total protein. Feed lot experiments in which the overall effects of rations are measured and in which protein is fed at fairly liberal levels may not bring out even fairly large differences in digestibility or utilization of the protein in the protein supplement.

SUMMARY

Nitrogen-balance experiments with lambs were conducted to determine the effect of heat treatment and oil extraction on the digestibility and utilization of soybean protein. The soybean feeds used in the experiments were raw soybeans, unextracted soybean flakes, solvent-process soybean oil meal, and heat-treated solvent-process soybean oil meal.

The rations, as fed, contained approximately 11 percent total protein on an air-dry basis, 10 percent being furnished by one of the soybean feeds and 1 percent by the low-protein basal ration. All nitrogen balances were of 10 days' duration, and the eight lambs in each experiment were fed each of the four rations tested in that particular experiment.

The apparent digestibility of protein for two experiments averaged 62.9 percent for the raw soybean ration, 69.0 percent for the solvent-process soybean oil meal ration, and 70.6 percent for the heat-treated solvent-process soybean oil meal ration. In a single experiment the protein in the soybean flake ration had a digestibility of 63.3 percent. The protein in a heat-treated soybean-oil meal ration with enough added soybean oil to equal that contained in the soybean ration had an average digestibility of 71.1 percent, as compared with an average of 73.1 percent for a similar ration without the added soybean oil, and 64.3 percent for the soybean ration.

The protein furnished by raw soybeans or unextracted soybean flakes had a significantly lower digestibility for lambs than the protein furnished by solvent-process soybean oil meal with or without special heat treatment. Apparently, this difference in digestibility was due mainly to the heat treatment given the meals since the addition of soybean oil did not significantly lower the digestibility of the protein.

The storage of total nitrogen intake in two experiments averaged 18.2 percent for the soybean ration, 23.7 percent for the solvent-process soybean oil meal ration, and 26.3 percent for the heat-treated solvent process soybean oil meal. In one experiment, the total nitrogen stored averaged 16.3 percent for the soybean flake ration as compared with 15.0 percent for the soybean ration. The storage of total nitrogen intake averaged 29.9 percent for the heat-treated soybean oil meal and 31.6 percent for a similar ration but with added soybean oil. The lambs stored significantly less of the total nitrogen intake when fed the soybean or the soybean flake ration than when fed any of the other rations. Further heat treatment of the solvent-process soybean oil meal did not result in a significant improvement as measured by storage of total nitrogen. The storage of total nitrogen was fully as high by the lambs when fed the soybean oil meal ration with added fat as when fed a similar ration without the added fat, and the storage was significantly higher than when the lambs were fed the soybean ration.

The difference in percentage of *total* nitrogen stored between the soybean or soybean flake ration and either of the solvent-process soybean oil meal rations was due chiefly to a lower digestibility. This was indicated by the fact that the differences in percentage of *digested* nitrogen stored between these rations were relatively smaller. The differences in biological values were so slight that they were not significant at all.

Contrary to data obtained with nonruminants, these data with lambs show that additional heat treatment of solvent-process soybean oil meal results in no appreciable improvement in the protein. The higher value of soybean oil meal protein, as compared with raw soybean protein, resulted mainly from higher digestibility rather than from greater efficiency in the utilization of the digested nitrogen.

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